

**A Method To Assess The Ecological Integrity
Of Urban Watersheds That Integrates
Chemical, Physical And Biological Data**

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Submitted to the Department of Civil and Environmental Engineering in
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Doctor of Philosophy in Environmental Engineering

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Abstract

An integrated method was developed to assess the ecological integrity of an urban watershed, and to evaluate the cumulative impacts of physical habitat degradation and metal-contaminated habitats upon its fish and macroinvertebrates. Sixteen sites in this watershed (the Aberjona watershed, in eastern Massachusetts) and 4 ecoregional reference sites (sites chosen to represent minimally-impaired conditions) were assessed. An innovative approach was used to evaluate metal contamination of macroinvertebrate epifaunal habitats (i.e., submerged vegetation, vegetation-covered rocks, overhanging bank vegetation, and undercut bank roots). Higher concentrations of As, Cr, Cu, Pb and Zn were measured in epifaunal habitats than in fine-grained sediments traditionally collected for risk assessments, and the ranking of sites by contamination level depended upon which sample type was used.

As predicted, the biological condition of fish and macroinvertebrate assemblages from minimally-contaminated sites varied from good to poor as habitat condition varied from good to poor. Biological degradation beyond that attributable to habitat degradation alone was observed, as predicted, at sites contaminated in excess of MacDonald's (1992) Expected Risk Median values. When the number of native fish species caught at a site was used as the measure of biological condition, and habitat condition was a simple function of stream depth and instream cover score, this predicted relationship was observed. Contamination of epifaunal habitats by As and Cr was reflected in elevated concentrations in whole white suckers. Twelve benthic metrics were used to characterize the biological condition of macroinvertebrate assemblages and to relate biological impairment to chemical and physical degradation (using linear regression and *t*-tests comparing site categories). Similar levels of biological impairment were observed at sites with severe physical *or* chemical degradation, or moderate chemical *and* physical degradation. Individual benthic metrics were not diagnostic of impairment type. An aggregate macroinvertebrate index was developed that was more sensitive to chemical and physical degradation than *any* individual metric, illustrating the strength of a multimetric index approach for detecting the cumulative impacts of physical habitat degradation and chemical contamination.

The method developed for the dissertation has a variety of applications for environmental protection programs and for ecological risk assessments.

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Figure 1.a
Aberjona Watershed Site Map

Sites Ab 1, Ab 2, Ab 3, Ab 4, Ab 5 and Ab 6 are located on the Aberjona river

Sites H1, H2, H3, H4, H5, H6, H7 and H8 are located in the Horn Pond subwatershed.

Sites Tr 1, Tr 2 and Tr 3 are located on tributaries of the Aberjona river

U S Geological Survey metric topographic maps - Boston North, Massachusetts (1985) and Reading, Massachusetts (1987) - were scanned into a computer and reduced in size. Each grid square is 1 square kilometer in area. Maps were compiled by the U.S.G.S. by photogrammetric methods from aerial photographs taken in 1978 and field checked in 1979.

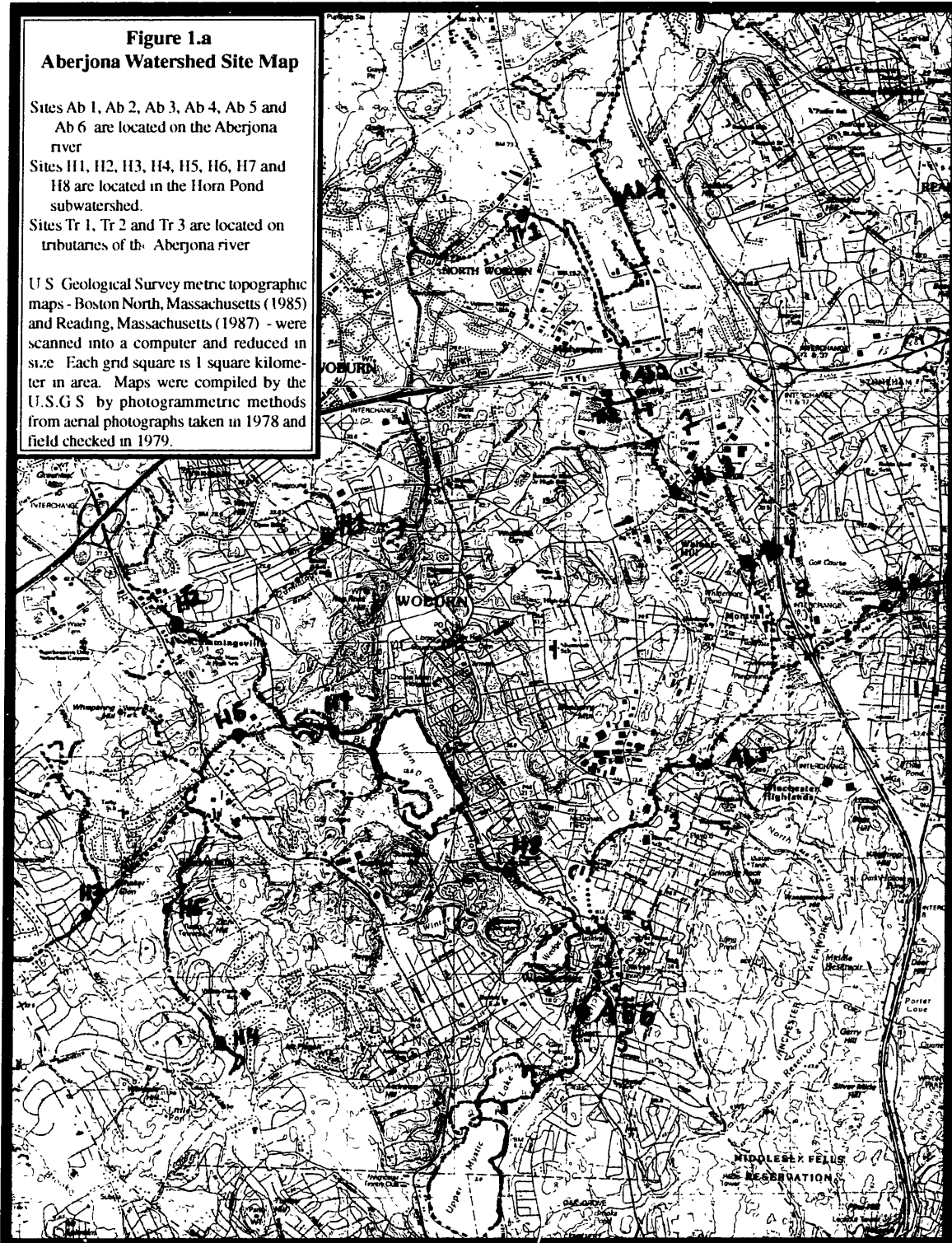
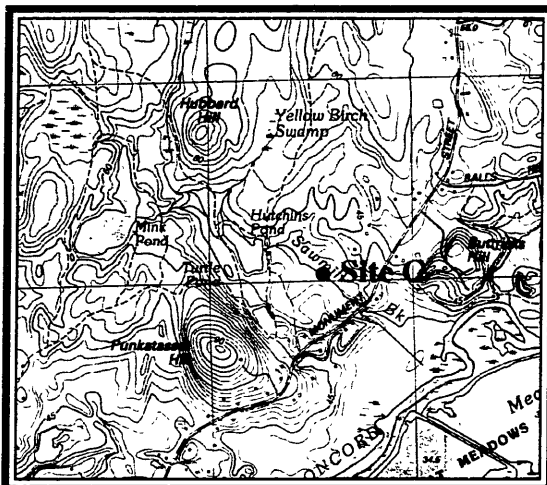
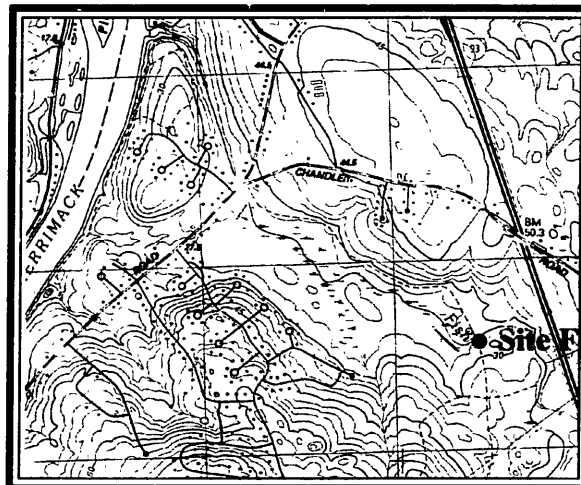


Figure 1.b Reference Site Maps

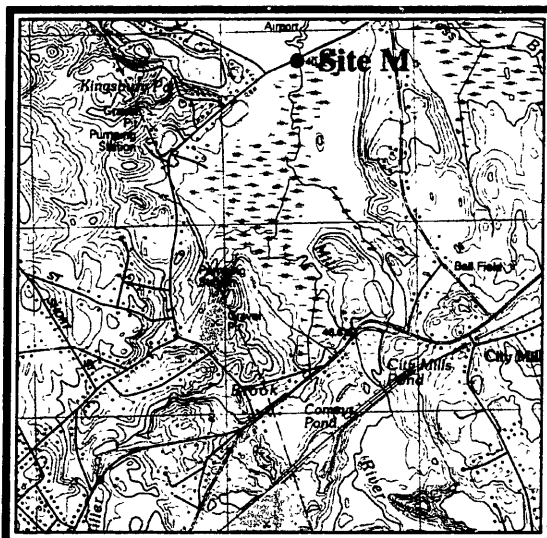
U.S. Geological Survey metric topographic maps (Maynard, Lawrence, Franklin and Framingham, MA, published in 1987) were scanned into a computer and reduced in size. Each grid square is 1 square kilometer in area. Maps were compiled by the U.S.G.S. by photogrammetric methods from aerial photographs taken in 1978 or 1981, and field checked in 1978, 1979 or 1982.



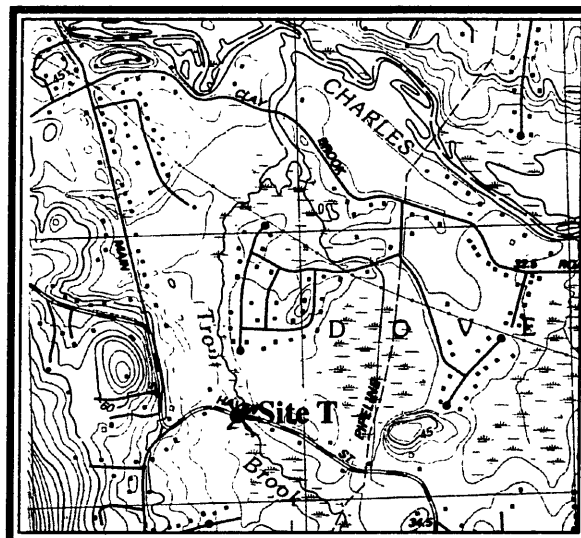
Site C is located on Sawmill Brook.
(Maynard, MA)



Site F is located on Mill River.
(Lawrence, MA)



Site M is located on Fish Brook.
(Franklin, MA)



Site T is located on Trout Brook.
(Framingham, MA)

Chapter 1:

Introduction

The objective of the Clean Water Act is to “restore and maintain the chemical, physical and biological integrity of the Nation’s waters” [FWPCA §101, §1251(a)]. Adapting Karr and Dudley’s (1981) definitions of biological and ecological integrity, ecological integrity is possessed by a system with chemical, physical and biological integrity that has the capability and actually does support and maintain a balanced, integrated “community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region.”

Although ecological integrity is the goal of the Clean Water Act, until recently it was rarely embraced by regulators or researchers (Angermeier and Karr 1994, Hughes & Noss 1992, Karr 1993); initially only chemical integrity was emphasized. In the 1970s, federal environmental regulations were narrow in focus, emphasizing the regulation of point source releases of sewage and toxic chemicals to surface waters (Rankin 1995). The effects of pollution upon biological communities in the environment were rarely studied directly; instead, emphasis was placed upon regulating the amounts of pollution being released to the environment and determining safe levels of exposure to toxic chemicals using laboratory organisms as surrogates for natural communities.

The narrow focus of federal environmental regulations in the 1970s limited their effectiveness (Rankin 1995, Karr 1991). Regulatory efforts to eliminate problems caused by chemical pollution were only partially successful because it proved impossible to characterize all the adverse effects of all the hazardous chemicals upon natural biological communities using only the laboratory toxicity approach (Thurston *et al.* 1979, Gosz 1980 from Karr 1981) and because nonpoint sources (e.g., urban and agricultural sources) of hazardous chemicals were largely left unregulated. Eutrophication and acid rain continued to threaten ecosystems. Little attention was given to protecting habitat from degradation by channelization, loss of riparian vegetation, sedimentation and other alterations (Rankin

1995, Hughes *et al.* 1990, Karr 1991). This loss of habitat quality has resulted in extinctions (Williams *et al.* 1989), local extirpations (Karr *et al.* 1985), and population reductions (Trautman 1981, Ohio EPA 1992) of fish species and other aquatic fauna (e.g., Williams *et al.* 1993) in the United States. (Rankin 1995) Consequently, although chemical pollution continues to be a problem in many freshwater stream ecosystems, many believe that habitat degradation is presently responsible for more ecological impairment than chemical pollution (Rankin 1995, Stevens 1993).

Although many states now include habitat assessment and biological surveys in their water resource protection programs (Rankin 1995, Davis *et al.* 1996, Southerland and Stribling 1995), these programs are generally not fully integrated with other programs. Different divisions of the same agency, or different divisions of different agencies, are responsible for hazardous waste sites, monitoring water quality, issuing pollution discharge permits, addressing nonpoint source urban and agricultural pollution, wetlands, protecting endangered species, and handling zoning permits. While there are good reasons to partition responsibilities, it is unfortunate that a common result is a lack of integration of chemical, physical and biological data. Establishing the linkages between chemical, physical and biological impairment enables managers to identify and remediate the primary threats to physical, chemical and biological integrity.

When ecological risk assessment protocols were first developed, they emphasized measuring and comparing contaminant concentrations in the field with concentrations that cause adverse effects in laboratory organisms. This method is still used as a way of screening sites for possible ecological risks, but recognition of the limitations of this approach has evolved in the last decade. The standard framework for ecological risk assessment presently advocated by the U.S. Environmental Protection Agency (U.S. EPA) consists of problem formulation, exposure assessment, effects assessment, and risk characterization (U.S. EPA 1992). Exposure assessment can consist of simply measuring contaminant concentrations in various media (water, sediments, a species' food), and effects

assessment can consist of literature review of laboratory toxicity studies. When the possibility of significant risk is indicated, however, additional analyses are often performed. These investigations may include field or laboratory studies, and may entail collection and analysis of tissue samples, bioassays, bioavailability studies, or a biodiversity study (DeSesso 1995). Durda (1993) recommends a triad approach: (1) bioassays (which determine toxicity of contaminated media to test organisms) establish toxicity, (2) physiological, biochemical, or chemical exposure biomarkers establish exposure, and (3) biological surveys measure the structural and functional characteristics of populations and communities at contaminated sites and compare them to reference sites.

The U.S. EPA has identified classes of chemical, physical and biological stressors (U.S. EPA 1994). Approaches have been developed to address nonchemical stressors. Nevertheless, most ecological risk assessments continue to focus on the risks of chemicals alone, even though most chemically contaminated sites also suffer from physical habitat impairment. In some cases, biological surveys are included in risk assessments that focus upon chemical risks. Chapman *et al.* (1992) urge the use of a sediment quality triad that includes measurements of chemical concentrations, toxicity testing, and biological surveys. This type of integrated approach is valuable because it links stressors to biological responses through a weight of evidence approach. In the current budget-conscious climate, being able to link chemical contamination to measurable ecological effects is necessary to justify the costs of remediation.

The problems of evaluating cumulative impacts of sediment contamination and physical habitat degradation, especially in urban/suburban systems, still pose challenges to researchers, risk assessors and environmental protection officials. When chemical risk is indicated by a preliminary screening of contaminant concentrations, I believe that biological surveys and habitat assessments should be included to adequately evaluate cumulative impacts before management actions are taken. This approach facilitates the interpretation of the biological survey data, allows chemical risks to be put in context with physical risks,

and facilitates effective remedial actions by ensuring that habitat degradation is considered. It is important to assess the physical habitat because a degraded habitat will prevent the attainment of healthy biological condition, even if enormous sums are expended to remediate chemical contamination.

The watershed concept is being advanced by the U.S. EPA as a framework for addressing the proper integration of data to resolve cumulative impacts. The watershed protection approach (WPA) is being used by the U.S. EPA and many states (including Massachusetts) as an organizing concept for integrating across agency programs and geographic area. The WPA has also provided an organizing concept for concerned citizens who have created watershed associations for the protection and restoration of their watersheds. The U.S. EPA is also engaged in developing watershed ecological risk assessment case studies at several locations around the nation (personal communication, William Farland, Director National Center for Environmental Assessment, U.S. EPA, Washington, D.C. and Patti Tyler Region 1, U.S. EPA, Lexington, Massachusetts). The framework for the WPA is in the development stage around the country. In this study, I implemented a WPA in the Aberjona Watershed.

Motivation for the Thesis

The Clean Water Act's objective of integrating chemical, physical, and biological components of environmental assessment and protection presents a formidable challenge to federal, state and local environmental protection agencies. The restoration and maintenance of chemical, physical and biological integrity involves identifying the primary threats to integrity, taking remedial actions to address those threats, monitoring improvements in chemical, physical and biological condition, and using monitoring information to adapt management actions to maximize ecological benefits.

The Thesis

An integrated method was developed to assess an urban watershed's chemical, physical and biological condition. The watershed that was selected as a case study for the development of the method is the Aberjona River Watershed (an urban watershed in the Boston Basin ecoregion of Massachusetts, Fig. 1.a). Within its 65 square kilometers, it contains two U.S. EPA Superfund sites and over a hundred state-listed suspected hazardous waste sites (Massachusetts Department of Environmental Protection [MA DEP] 1993). Its stream sediments are extensively contaminated with high concentrations of toxic metals. Although it seemed likely from the outset of the study that chemical contamination was seriously impacting biological integrity, I hypothesized that habitat degradation could be an even more significant cause of impaired biological integrity within the watershed.

The assessment of the Aberjona watershed involved developing a reference condition for the Boston Basin ecoregion, which was difficult because minimally-impacted areas are rare in this ecoregion (chapter 2). Fish and macroinvertebrate assemblages in urban streams are typically subjected to a variety of chemical and physical stressors. Thus, I assert that field investigations of contaminant impacts (e.g., for research, management, and/or ecological risk assessment) need to consider differences in habitat quality between sites. In the Aberjona watershed, sites with varying degrees of chemical and physical stress were compared to sites with minimal stress (ecoregional reference sites), and to each other. The physical habitat index (composed of ten habitat parameters, each scored from 0 to 20) that is used by the MA DEP (Tetra Tech 1995) was used to assess physical habitat degradation in the Aberjona watershed (chapter 2, Appendix A). The exposure of macroinvertebrates to contaminants was evaluated by sampling submerged vegetation, vegetation-covered rocks, overhanging bank vegetation, and undercut bank roots (chapter 2). Higher concentrations of As, Cr, Cu, Pb and Zn were found in these epifaunal habitats than in the fine-grained sediments traditionally collected for risk assessments, and the ranking of sites by contamination level depended upon which sample type was used.

The conceptual model of Barbour *et al.* (1997) asserts that biological condition varies from good to poor as habitat condition varies from good to poor when chemical contamination is minimal, and that biological degradation beyond that attributable to habitat degradation is observed when contaminants are adversely affecting biological assemblages. Long and MacDonald (1992) predict that contaminants adversely affect biological assemblages when the concentrations of contaminants in sediments exceed Expected Risk Median values (Long and Morgan 1990, Long and MacDonald 1992, MacDonald 1992). The predictions of Barbour *et al.* (1997) and Long and MacDonald (1992) were confirmed in the Aberjona watershed when the number of native fish species caught at a site was used as the measure of biological condition, and habitat condition was a simple function of stream depth and instream cover score (a component of the physical habitat index) (chapter 3). Fewer native fish species were caught at sites with elevated concentrations of As, Cr, Cu, Pb and Zn in the sediments and epifaunal habitats than were caught at sites with comparable habitat condition and minimal contamination. In addition, contamination of sediments and epifaunal habitats was reflected in elevated concentrations of As and Cr in whole white suckers (chapter 3).

Twelve benthic metrics were used to characterize the biological condition of macroinvertebrate assemblages and to relate biological impairment to chemical and physical degradation (using linear regression and *t*-tests comparing site categories) (chapter 4). Similar levels of biological impairment were observed at sites with severe physical *or* chemical degradation, or moderate chemical *and* physical degradation. Individual benthic metrics were not diagnostic of impairment type. An aggregate macroinvertebrate index was developed that was more sensitive to chemical and physical degradation than *any* individual metric, illustrating the strength of a multimetric index approach for detecting the cumulative impacts of physical habitat degradation and chemical contamination (chapter 4).

The potential for various aspects of the dissertation to contribute to environmental management are numerous (Table 5.1, chapter 5). The implications of the dissertation for the remediation of the Aberjona watershed (Table 5.2), and the feasibility of environmental protection agencies adopting the method developed by the dissertation, are discussed in chapter 5.

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Chapter 2:
Diagnosing Chemical and Physical Causes of Aquatic Life Impairment:
Results from the Aberjona Watershed, MA

C.E. Rogers, M.T. Barbour, D. Brabander, and H.F. Hemond

Introduction

In many industrial, urban, and suburban areas, anthropogenic releases of toxic chemicals to surface waters have declined, but high concentrations of contaminants persist in the sediments. Sediment contamination in these areas is usually accompanied by physical degradation of stream habitat by channelization, sedimentation, and alteration of stream banks (especially the removal of vegetation). Sediment contamination impairs the biological integrity of aquatic assemblages (e.g., fish and macroinvertebrates), but physical habitat forms the template within which biological communities develop and limits their biological potential (Southwood 1977). Thus, restoration of a degraded stream's potential to support aquatic assemblages often will require restoration of physical as well as chemical integrity.

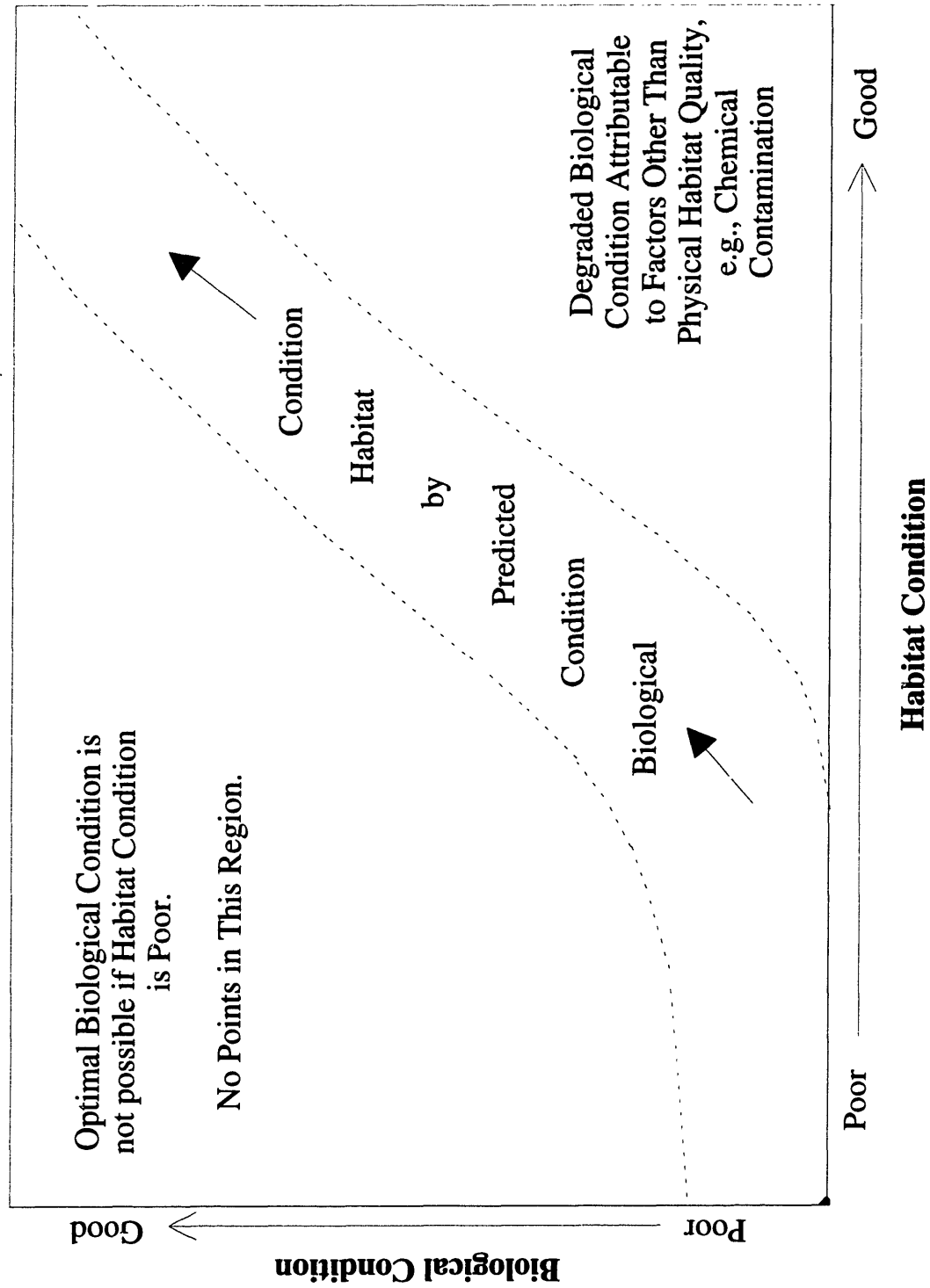
The National Status and Trends Program (NSTP) Approach for assessing the risks of contaminated sediments was developed for the National Oceanic and Atmospheric Administration (NOAA) by Long and Morgan (Long and Morgan 1990, MacDonald 1992, Long and MacDonald 1992). The NSTP Approach identifies three effect ranges: no-effects ($\text{conc} < \text{ERL}$), possible-effects ($\text{ERL} < \text{conc} < \text{ERM}$) and probable-effects ($\text{conc} > \text{ERM}$), in which the ERL (Expected Risk Low) is the lower 10th percentile concentration associated with toxic effects (using laboratory spiked-sediment bioassays, equilibrium partitioning and field studies) and the ERM (Expected Risk Median) is the median concentration associated with toxic effects (Long and Morgan 1990, MacDonald 1992, Long and MacDonald 1992). The approach presumes that the potential for toxicity is relatively high in areas where numerous chemicals exceed the ERM, and that the potential for toxicity is low in areas

where none of the chemical concentrations exceed the ERL. This approach can be used to estimate the likelihood of adverse effects of contaminated sediments upon biological assemblages, but without physical habitat assessment, it may be difficult to link chemical stressors to biological responses.

A graphical framework for relating biological condition to physical habitat degradation and chemical contamination was proposed by Barbour and Stribling (1991) and further elaborated by Barbour *et al.* (1996, 1997); their framework is represented in Figure 2.1. They hypothesized that biological condition has a predictable relationship (e.g., a sigmoid curve) with physical habitat condition: increasing biological quality is associated with increasing habitat quality. Biological degradation in the presence of good habitat quality can be attributed to something other than habitat quality (Barbour and Stribling 1991, Barbour *et al.* 1996a, Barbour *et al.* 1996b). In cases of biological degradation in the presence of intermediate habitat quality, distinguishing between habitat effects and other stressors is difficult (Barbour *et al.* 1997). Thus, a stressor such as sediment contamination could be expected to lower biological condition if habitat condition is good, but the effects might be difficult to distinguish from habitat effects if habitat condition is poor or intermediate.

Ecological risk assessment techniques are commonly employed (e.g., by state and federal regulatory authorities, consultants for companies responsible for hazardous waste sites, etc.) to evaluate the risk of chemically-contaminated sediments. Unfortunately, ecological risk assessments do not routinely include physical habitat assessments, and often do not include biological surveys. Without biological surveys, it is impossible to document a stressor's impact on biological assemblages. Even if biological surveys are included, without habitat assessment, it is not possible to distinguish impacts caused by chemical contamination from the impacts of habitat degradation. Furthermore, actions taken to remediate chemical contamination may fail to produce significant ecological benefits if habitat degradation is not addressed.

**Figure 2.1 Conceptual Model of Barbour *et al.* (1997)
Relationship of Biological Condition to Habitat Condition**



Rapid bioassessment protocols (e.g., Barbour *et al.* 1997, Tetra Tech 1995) exist for surveying aquatic assemblages and assessing physical habitat quality, although additional development of biocriteria is needed to adequately cover urban systems (Gibson *et al.* 1996). In many states, programs designed to address chemical contamination of sediments function independently from programs that survey aquatic assemblages and measure physical habitat degradation. Different divisions of the same agency, or different divisions of different agencies, are responsible for managing hazardous waste sites, monitoring chemical water quality, performing habitat assessments and biological surveys, issuing pollution discharge permits, addressing nonpoint source urban and agricultural pollution, protecting wetlands, protecting endangered species, and regulating land use (e.g., through zoning permits). While there are good reasons to partition responsibilities, it is unfortunate that a common result is a lack of integration of chemical, physical and biological data. Establishing the linkages between chemical, physical and biological impairment is essential in enabling managers to identify and remediate the primary threats to physical, chemical and biological integrity.

Environmental assessment and protection programs in the Aberjona watershed (defined as the area drained by the Aberjona River, Fig. 1.a) illustrate this point. The regulatory responsibilities described above are performed by various divisions within the Massachusetts Department of Environmental Protection (MA DEP), the U.S. Environmental Protection Agency (EPA), and by local authorities (e.g., Conservation Commissions of Woburn and Winchester). Two (EPA National Priority Listed) Superfund sites are located on the Aberjona River. Concentrations of toxic chemicals in the Aberjona River have declined, but high concentrations of contaminants persist in the sediments. Human health and ecological risk assessments are in progress to measure the impacts of chemical stressors from the Superfund sites (personal communication, Mary Garren and Joseph LeMay, EPA Region 1), and the MA DEP is overseeing limited risk assessment activities for the numerous non-Superfund waste sites in the watershed (over 100 sites are under

investigation) (MA DEP 1993). These risk assessments will not include physical habitat assessment or biological surveys (although species have been/will be caught for contaminant analysis).

Discussion with regulators and citizens (e.g., members of the Mystic River Watershed Association) revealed an opportunity to design a research program that could facilitate environmental protection in the Aberjona watershed, and contribute to the diagnosis of chemical and physical causes of cumulative impacts to aquatic life in urban systems. Our research program integrates *chemical* assessment (measurement of metal concentrations in sediments, epifaunal habitat samples, and ground whole fish), *physical* assessment (habitat assessment following the MA DEP protocols), and *biological* surveys (collection and identification of fish and macroinvertebrate assemblages). We hypothesized that a relationship between biological impairment (reflected by fish and macroinvertebrate assemblages) and physical habitat condition would be found using a multimetric evaluation of chemical, physical and biological condition. We further hypothesized that description of this relationship would facilitate an understanding of the contribution of chemical contamination to biological impairment. This paper describes the methods and the results of the chemical and physical assessment of the Aberjona watershed.

Methods

Site Selection

Seventeen locations in the watershed were chosen for study (Fig. 1.a): 6 are located along the Aberjona River from its source to its end in the Mystic Lakes; 8 sites are located throughout the Horn Pond subwatershed that carries water from the western part of the Aberjona watershed to the Aberjona River between Aberjona River sites Ab 5 and Ab 6; and 3 sites are located on the three largest remaining tributaries to the river. Locations were chosen from a combination of U.S. Geological Survey (U.S.G.S.) maps (Reading, MA, 1987 and Boston, MA-North, 1985), street maps, and site visits. All sites are in walking

distance (usually upstream) of road crossings. Four additional locations were chosen as “minimally-impaired” sites.

Selection of “Minimally-Impaired” Sites

The entire Aberjona watershed is influenced by heavy human-related development, and the watershed is part of the Boston Basin ecoregion (Griffith *et al.* 1994). The least-disturbed stream reach in the Aberjona watershed was selected as a watershed reference site. This site is located in the Horn Pond recreational area, and was named site H7 (Fig. 1a).

Additional reference sites were sought within the ecoregion (e.g., within least-disturbed areas of the Charles River Watershed and the Concord River Watershed). The criteria for the reference sites were: (1) relatively undeveloped headwaters, (2) no evidence of human alteration of the physical habitat, (3) wide (> 18m) vegetated riparian zones, and (4) no evidence of pollution (Hughes *et al.* 1990). Fifty candidate reference locations were selected from U.S.G.S. maps and from consultation with knowledgeable resource managers and scientists (personal communication with Scott Socolosky of MIT, the Charles River Watershed Association, the U.S. Army COE, U.S.G.S., the Massachusetts Department of Environmental Protection, and the U.S. EPA New England Regional Lab in Lexington, MA). These locations were selected because their geological and hydrological properties (stream order, drainage area and gradient) were similar to the sites in the Aberjona Watershed (U.S.G.S. maps: Reading, MA, 1987 and Boston, MA-North, 1985) and because minimal disturbance was suggested by available information. Four sites (named C, F, M and T) were determined to be of acceptable quality, and were chosen as “minimally-impaired” sites (Fig. 1.b).

Habitat Assessment

Physical habitat assessment was performed at 17 test sites and 4 reference sites (Fig. 1) according to the protocols used by the Massachusetts Department of Environmental Protection (Tetra Tech 1995) modified for slow-flow, low gradient streams. Ten habitat parameters (instream cover, epifaunal substrate, embeddedness, channel alteration, sediment deposition, variety of velocity-depth combinations, channel flow status, bank vegetative protection, bank stability, and riparian vegetative zone width) were scored from 0 to 20 (see Appendix A). Stream width, depth, and velocity were measured. The following water characteristics were also measured: the concentration of dissolved oxygen (YSI Model 57 Oxygen Meter), the temperature (YSI Model 57 Oxygen Meter), the pH (Jenco Analog pH Meter 611), and conductivity (Cole Parmer Conductivity Meter 1481-60). Characteristics of the substrate (% composition of various organic and inorganic components), and the percentage of sampling reach that is shaded (% shading) by overhanging vegetation were visually estimated.

Sample Collection for Metal Analysis

After visual determination of the range of sediment grain size and organic content, areas of fine-grained, organic-rich sediments were collected at 17 test sites and 4 reference sites (Fig. 1) using a Russian corer. The torpedo-shaped, aluminum corer head was gently inserted into the top 2-3 cm of sediment and twisted 180° to capture the sample. The corer was pulled out of the sediment, twisted again to expose the sample, and sediment was scooped off the corer with a teflon spatula and stored in glass jars.

Epifaunal habitats (submerged vegetation, vegetation-covered rocks, overhanging bank vegetation, and undercut bank roots) were sampled and stored in glass jars. Watercress was sampled at sites Ab 1, H4, H5 and Tr 2. Submerged vegetation was sampled at sites Ab 3, H7 and T. Undercut bank roots were sampled at sites Ab 2, Ab 5, Ab 6, H3, H8 and Tr 3. Vegetation was scraped from rocks at sites H2, H3, H6, C and F.

Silty overhanging bank vegetation was sampled at site Ab 4. A mixture of watercress and submerged vegetation was sampled at site Tr 1 and a mixture of emergent and overhanging bank vegetation was sampled at site M.

Jars containing sediment and habitat samples were immediately placed on ice and kept refrigerated (12 hours to 2 days) until they were dried at 85°C to a constant weight (approximately 1-2 days).

Metal Analysis

Five (5.0) grams of each dried sample were pulverized in a Spex Mixer cartridge with a silicon carbide ball for 5 minutes. One-half gram (0.5 g) of copolywax (TM) binder was added to the sample and mixed again for 1 minute. This mixture was poured into a 31 mm diameter aluminum sample cup and pressed for one minute using 12 metric tons of force in an evacuable die. Elemental concentrations in pellets were analyzed by X-Ray Fluorescence (XRF) using a Philips PW1480 wavelength dispersive XRF and Uniquant data processing software.

Toxic Unit Analysis

Concentrations of As, Cr, Cu, Pb and Zn were normalized by sediment quality benchmarks (Table 2.1, Expected Risk Low (ERL) and Expected Risk-Median (ERM) values, Long and Morgan 1990 updated by MacDonald 1992). The resulting risk ratios (environmental concentration/ benchmark concentrations) are dimensionless, and are referred to as toxic units. Ratios in excess of 1 occur when concentrations in sediments are higher than benchmarks.

Table 2.1. ERL and ERM Values for As, Cr, Cu, Pb and Zn (MacDonald 1992)

	As	Cr	Cu	Pb	Zn
ERM (ppm)	70	370	270	223	410
ERL (ppm)	8.2	81	34	46.7	150

Results

Table 2.2.a. Analysis of Precision

The standard error calculated by Uniquant (Uniquant 1992) for As, Cr, Cu, Pb and Zn primarily consists of counting statistical error and systematic errors in the corrections for background and line overlaps. This table presents the average concentration and standard error for each element for each set (sediment and habitat) of samples. CONCENTRATIONS AND STANDARD ERRORS ARE GIVEN IN PPM, DRY WEIGHT.

Element	Av. Value of Sediment Samples	Av. (Standard Error) -Sed	Av. % Standard Error of Sample	Av. Value of Habitat Samples	Av. (Standard Error) -Hab	Av. % Standard Error of Sample
As	144 (19 non-	12 detects)	8 %	336 (16 non-	21 detects)	6 %
Cr	152	9	6 %	219	12	5 %
Cu	93	6	6 %	115	7	6 %
Pb	185	10	5 %	181	9	5 %
Zn	343	17	5 %	657	30	5 %

Table 2.2.b. Analysis of Accuracy

NIST standard reference material #2709 (San Joaquin soil) was used to verify that the instrument was functioning properly. The average and standard deviation of 15 measurements (covering the period when samples were analyzed) of the concentrations of As, Cr, Cu, Pb and Zn are compared to the concentrations and standard deviations reported by NIST for this reference material.

Element	Av. Measured NIST	StDev of Measured NIST	Reported NIST	Reported StDev NIST	Ratio of Measured /Reported
As	nondetect				
Cr	131	10	130	4	1.01
Cu	42	3	35	1	1.20
Pb	19	4	19	1	1.00
Zn	85	8	106	3	0.80

Total habitat scores were in the optimal range (150-200) for reference sites (H7, C, F, M, T) and for site Ab 3 (Fig. 2.2). Other sites located on the Aberjona River or in the Horn Pond subwatershed had scores in the marginal (50-100) to suboptimal (100-150) range; all tributary sites had scores in the marginal range. The sites with the greatest overall degradation are sites Ab 5, Ab 6, Tr 1, Tr 2, H1, H2 and H8. The four minimally-impaired reference sites received excellent scores for all physical habitat parameters except sediment deposition and embeddedness. The lower scores for these two parameters were representative of the general condition of the Boston Basin ecoregion, and of the Aberjona watershed, in particular, which was characterized by slow-flowing streams with soft substrates.

Low instream cover and epifaunal substrate scores at several sites were indicative of poor availability and diversity of fish and macroinvertebrate habitat at these locations (Fig. 2.3a, Table 2.3). Suboptimal scores for sediment deposition and embeddedness at most reference sites reflected the character of the Boston Basin ecoregion (Fig. 2.3b, Table 2.3). We were not able to find any reference sites with optimal scores for all parameters. Sandy and/or silty substrates were common in the slow-flowing streams of the Aberjona watershed and the reference areas. Sites Tr 1 and H1 had significantly lower scores, which are indicative of serious impairment. Stream channel parameters reflected the urbanization of the Aberjona watershed: Aberjona watershed sites tended to have a greater degree of channel alteration, a smaller variety of velocity-depth combinations, and lower channel flow status than reference sites (Fig. 2.3c, Table 2.3). Sites Ab 3 and H7 had higher scores than other Aberjona watershed sites, a pattern that held for most habitat parameters. Riparian vegetative zone widths were narrow (0-5 m) at many sites in the Aberjona watershed, while they were of optimal width (> 18 m) at all reference sites (Fig. 2.3d, Table 2.3). At many sites, the riparian zone was not merely narrow, but also poorly vegetated (Fig. 2.3d, Table 2.3).

Figure 2.2. Summed Habitat Scores

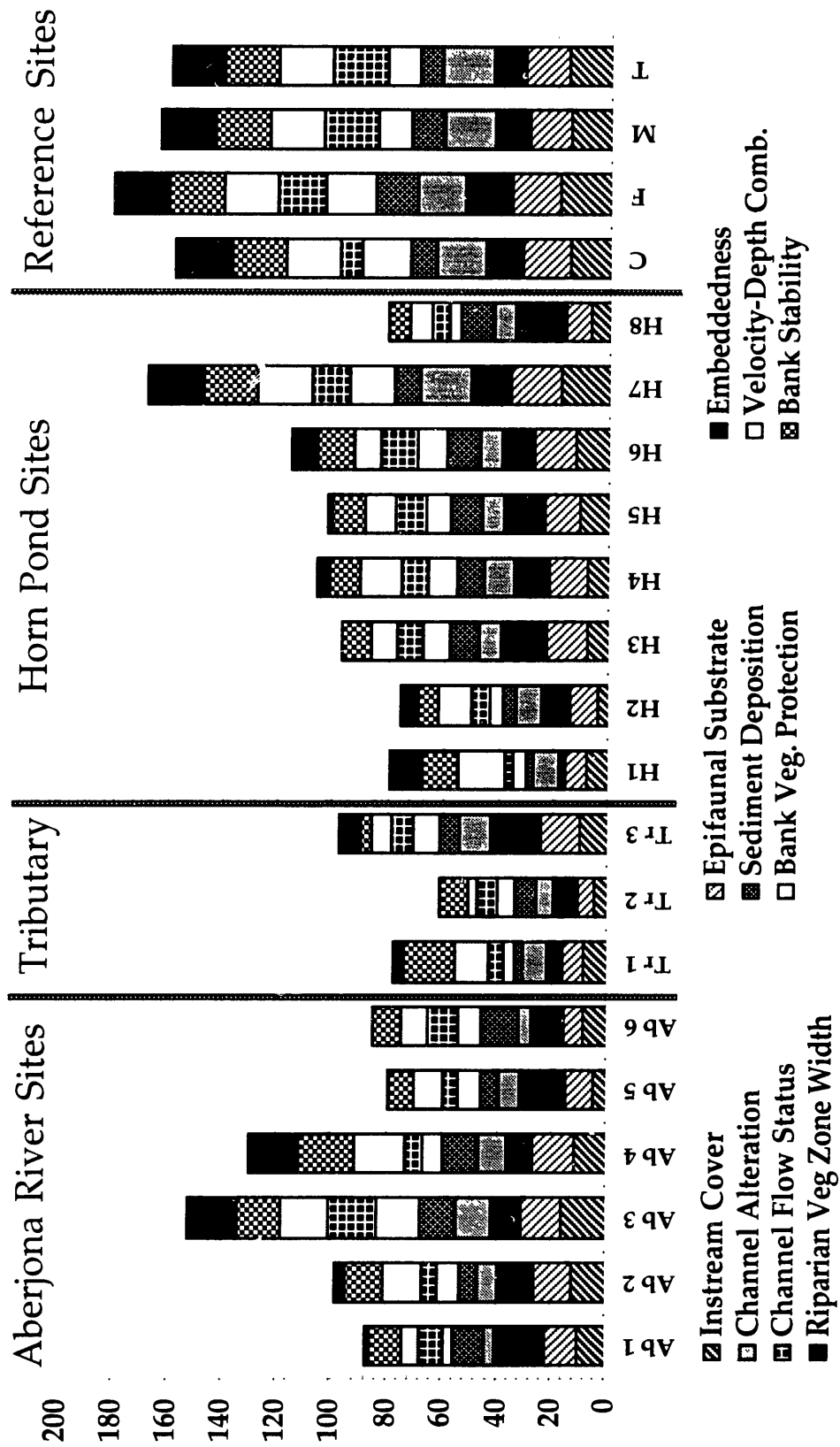


Figure 2.3. Scored Habitat Parameters

Figure 2.3.a. Instream Cover and Epifaunal Substrate

Figure 2.3.b. Sediment Deposition and Embeddedness

Figure 2.3.c. Stream Channel Parameters

Figure 2.3.d. Stream Bank Parameters

Figure 2.3.a. Instream Cover & Epifaunal Substrate

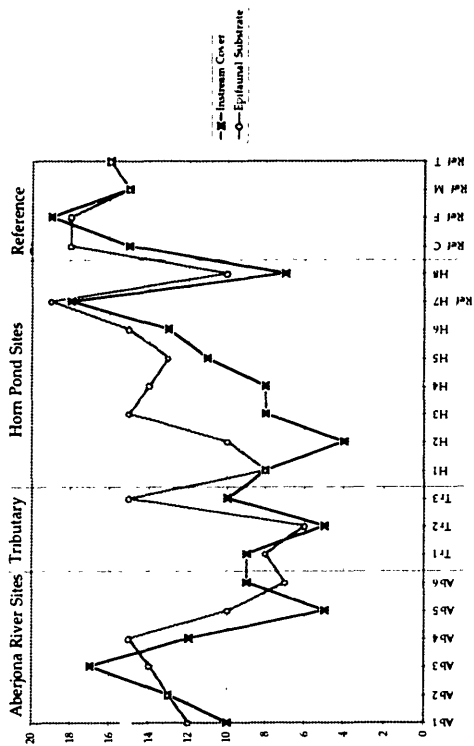


Figure 2.3.b. Sediment Deposition & Embeddedness

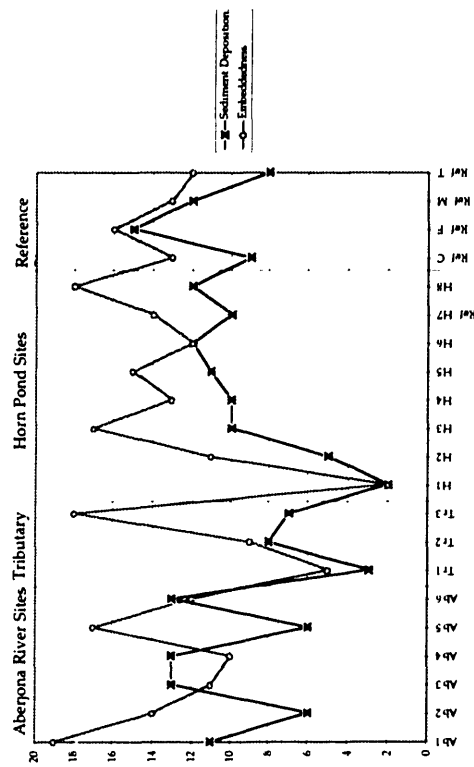


Figure 2.3.c. Stream Channel Parameters

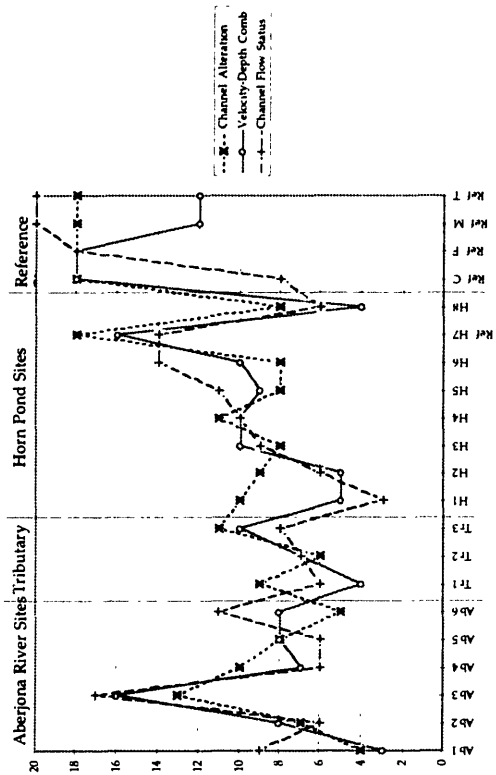


Figure 2.3.d. Stream Bank Parameters

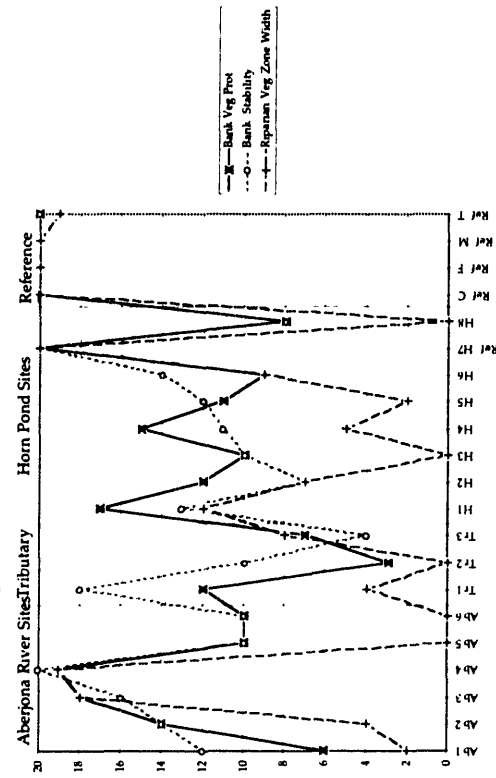


Table 2.3 Habitat Assessment Results

<u>Parameter</u>	<u>Relevance of Parameter</u> (Tetra Tech, 1995)	<u>Discussion of Impacted Sites</u>
<u>Instream Cover</u>	Fallen logs and branches, large rocks, and undercut banks at reference sites provide fish with a large number of niches for feeding, laying eggs, and refugia. Without these niches, fish diversity decreases and the potential for recovery following disturbance decreases.	Instream cover scores at Horn Pond sites H2, H3, H4, and H5, and sites Ab 5 and Tr 2 were very low, making it difficult for these sites to support diverse fish assemblages. Sites H2, H3, H4 and Tr 2 were unlikely to support large fish assemblages anyway, because of their small size, but Ab 5 is part of the Aberjona River and its ability to support fish was probably greatly reduced by habitat impairment.
<u>Epifaunal Substrate</u>	Snags, submerged logs, and other hard substrates provide habitat for macroinvertebrates.	H2, H8, Tr 1, Tr 2, and Ab 5 and 6 had reduced ability to support macroinvertebrates.
<u>Embeddedness</u>	Embeddedness, caused by the deposition of fine sediments, reduces the availability of desirable fish and macroinvertebrate habitat.	Embeddedness severely reduced habitat availability at Tr 1; the stream bottom, all vegetation, and all rocks, snags and branches were coated in a thick layer of fine grained material (silt and mud). Tr 2's stream bottom consisted mostly of sand, fine pebbles and mud, thus it also had a low embeddedness score.
<u>Sediment Deposition</u>	Sediment deposition results from large-scale movement of sediment and creates an unstable environment for many organisms.	Fine grained materials can be deposited on macroinvertebrates, fish eggs and plants; this was probably adversely affecting organisms at Tr 1.
<u>Channel Alteration</u>	Channelization reduces natural habitat for fish, macroinvertebrates and plants and increases scouring.	Channelization was particularly severe at sites Tr 2, Ab 1, Ab 2, and Ab 6.
<u>Velocity-Depth Combinations</u>	Frequency of riffles, or variety of velocity-depth combinations, is a measure of a stream's ability to provide and maintain diverse and stable aquatic environment and its ability to handle surges in flow during storms.	Biological assemblages at sites H2, H8, Tr 1 and Ab 1 were probably impaired by the homogeneity of velocity and depth profiles at these sites.

Channel Flow Status	Channel flow status is important because it determines whether habitat (such as cobbles, logs, snags, overhanging bank vegetation and undercut bank roots) are submerged (available for aquatic fauna) or exposed to the air (unavailable).	Low scores for sites Tr 1, Tr 2, Tr 3, H2, H8, Ab 2, Ab 4, and Ab 5. Site H8 drains the entire Horn Pond subwatershed, so it should have had a greater flow than H7. Instead, the stream was shallow in October 1995 and August 1996, and was completely dry in large sections in August 1997. This was because flow was diverted from this site by pumping from aquifers upstream, in the Horn Pond area. As a result, during low flow conditions fish became stranded, and macroinvertebrate habitat was exposed.
Bank Vegetative Protection	Bank vegetation provides habitat (such as submerged overhanging bank vegetation) and organic inputs (leaves provide food for shredders and other macroinvertebrates) and its roots hold soil in place and prevent erosion.	Tr 2 had low scores for bank vegetative protection, bank stability, and riparian vegetative zone width.
Bank Stability	Eroded banks lack vegetative protection and contribute to sediment deposition.	Tr 3, H2 and H8 had the most serious problems with bank stability.
Riparian Vegetative Zone Width	Wide riparian zones provide a buffer for pollutants, control erosion, and provide habitat.	Riparian vegetative zone width scores were zero at five sites and below 10 for all sites except reference sites and Ab 3 and Ab 4.
Temperature	Unvegetated stream banks have less canopy cover, thus less shade, thus water temperatures may be increased.	Aberjona watershed sites did not have higher water temperatures than reference sites.
Dissolved Oxygen Concentration	Decreased concentrations of dissolved oxygen can occur if pollution contributes to a high chemical and/or biological oxygen demand. Decreased concentrations of dissolved oxygen are harmful to fish and some macroinvertebrates.	Sites Ab 1 and H1 had lower concentrations of dissolved oxygen than reference sites. Other Aberjona watershed sites had comparable, and sometimes higher concentrations of dissolved oxygen.
pH, conductivity	Increases in alkalinity (higher pH) and conductivity can occur with urbanization.	Reference sites had lower pH and conductivity than Aberjona watersheds. The differences were small and are hard to see in the figure.

Water temperature had the same range for Aberjona watershed sites as for reference sites (Fig. 2.4a). Conductivity and pH were somewhat lower, and dissolved oxygen concentrations were nearly in the same range (Fig. 2.4a). Inorganic and organic sediment composition varied greatly between sites (Fig. 2.4b, 4c), but Aberjona watershed sites tended to have greater percentages of gravel and sand and lower percentages of organic material than reference sites. Canopy cover varied greatly within all site categories (Fig. 2.4d). Stream velocity was fairly constant among Aberjona River sites, but varied greatly among other site categories (Fig. 2.4f).

Reference sites had the lowest concentrations of As, Cr, Cu, Pb and Zn (Fig. 2.5). The highest concentrations of all metals occurred in the Aberjona River sites. The concentrations in the Aberjona River sites tended to be significantly higher in habitat samples than in sediment samples.

Concentrations of arsenic in both sediment and epifaunal habitat samples were below detection limits (20 ppm) at all non-Aberjona River sites (Fig. 2.5a). Arsenic concentrations as high as 300 ppm (sediment) and 800 ppm (habitat) occurred at the Aberjona River sites. Cr concentrations were low at all sites except Tr 1, and Ab 2-6 (Fig. 2.5b). The highest concentrations of Cr occurred at sites Ab 3 and Ab 4, peaking at almost 700 ppm in sediment samples, and at almost 1200 ppm in the epifaunal habitat samples. Concentrations of Pb in sediment samples were variable across site types (Fig. 2.5d). Concentrations of Pb in epifaunal habitat samples of the Aberjona River were higher than concentrations in other habitat samples. Zn concentrations were highest in Aberjona River sites Ab 2-6. Concentrations in the sediment were as high as 1400 ppm, and in the habitat samples were as high as 2000 ppm (Fig. 2.5e).

Figure 2.4. Selected Physico-chemical Parameters

Figure 2.4.a. Water Quality Parameters

Figure 2.4.b. Inorganic Sediment Composition

Figure 2.4.c. % of Sediment Surface Covered by Organic
Mud

Figure 2.4.d. % of Canopy Cover

Figure 2.4.e. Stream Width and Depth

Figure 2.4.f. Stream Velocity

Figure 2.4.a. Water Quality Parameters

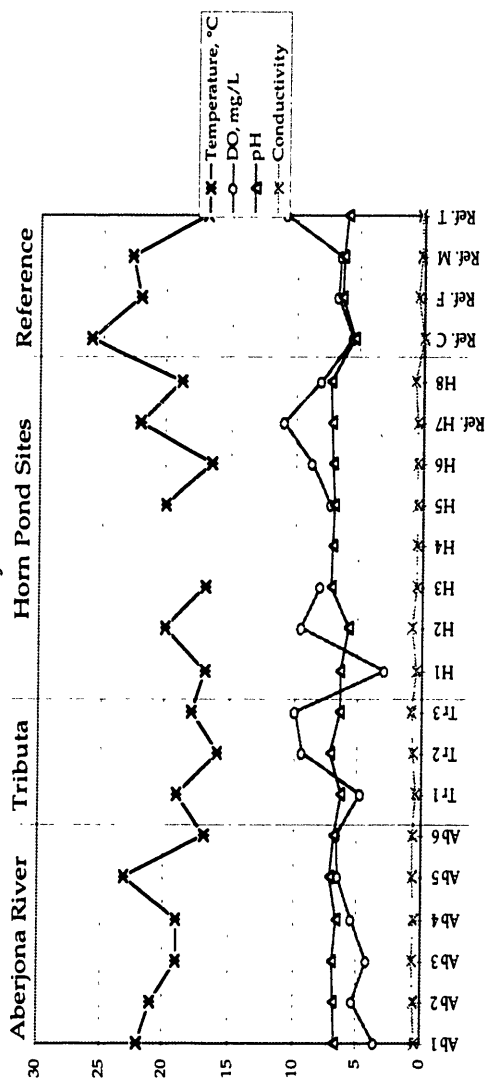


Figure 2.4.b. Inorganic Sediment Composition

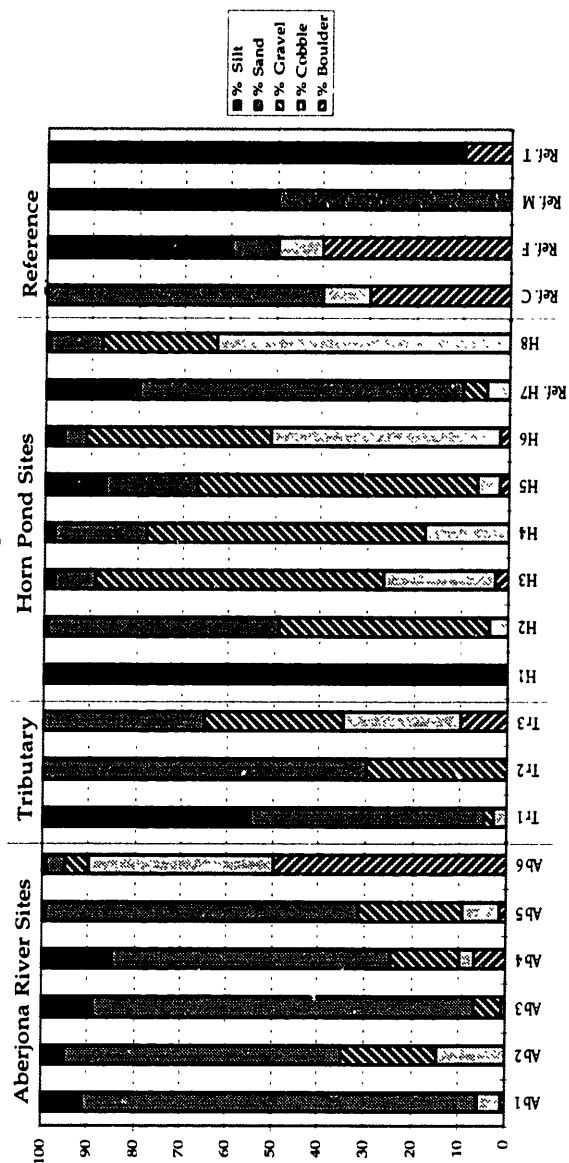


Figure 2.4.c. % of Sediment Surface Covered by Organic Material

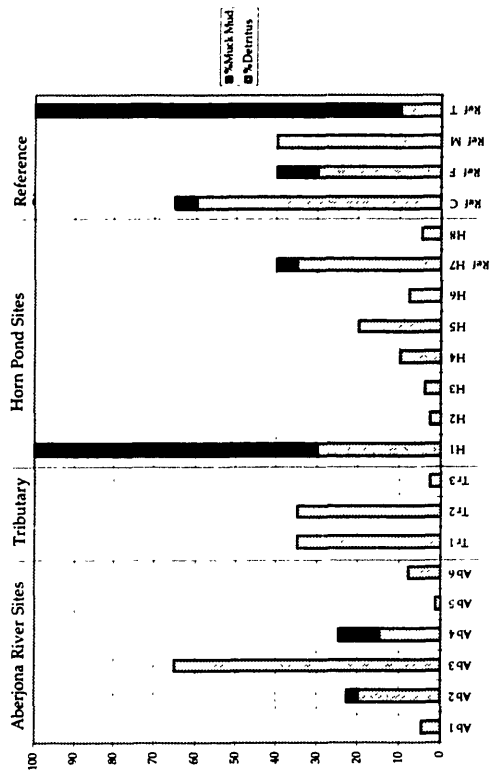


Figure 2.4.d. % Canopy Cover

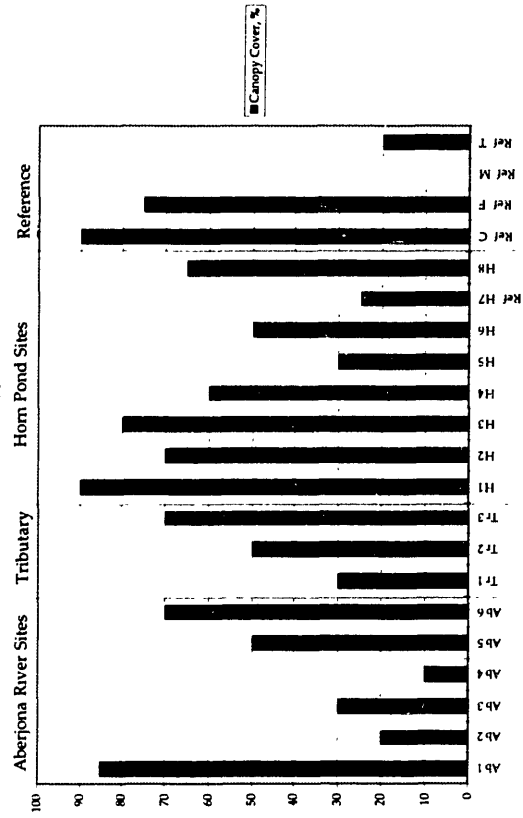


Figure 2.4.e. Stream Width & Depth

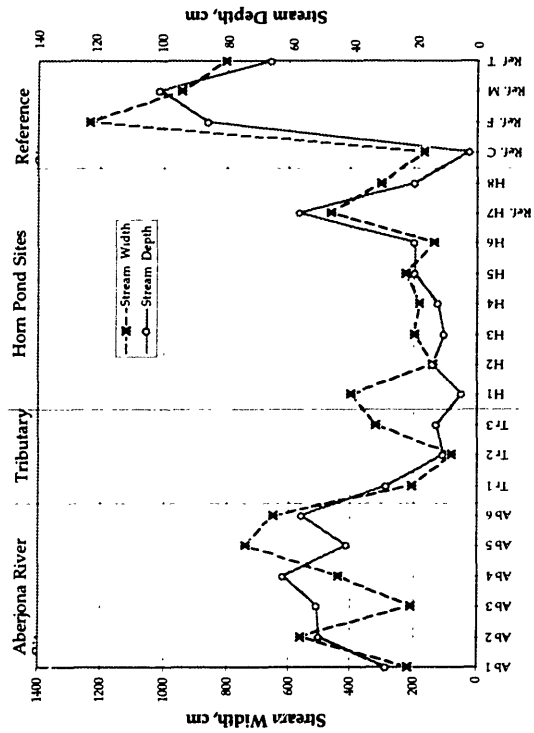


Figure 2.4.f. Stream Velocity

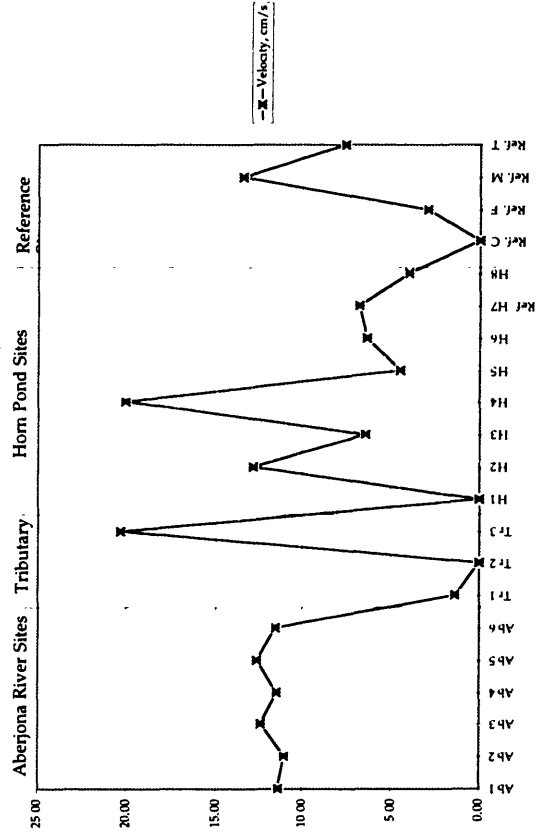


Figure 2.5 Concentrations of As, Cr, Cu, Pb and Zn in Sediments and Epifaunal Habitat Samples

Fig. 2.5.a.1. [As] in Sediments

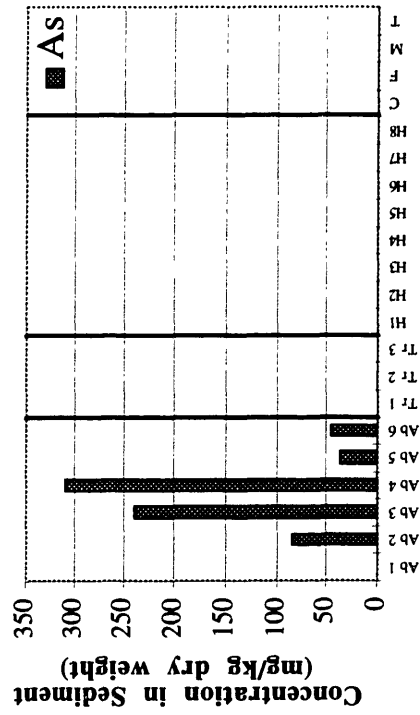


Fig. 2.5.a.2. [Cr] in Sediments

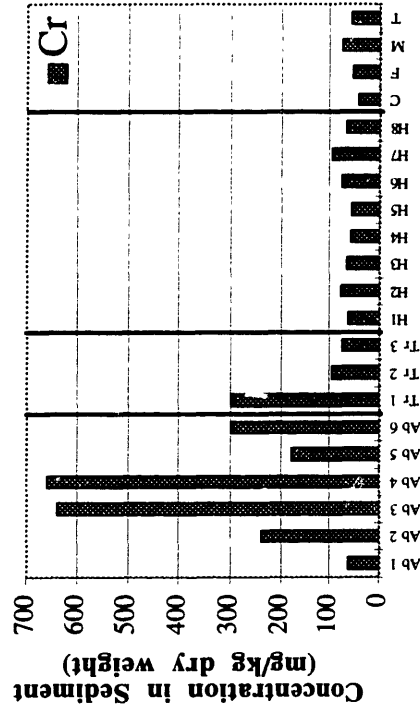


Fig. 2.5.b.1. [As] in Epifaunal Habitat

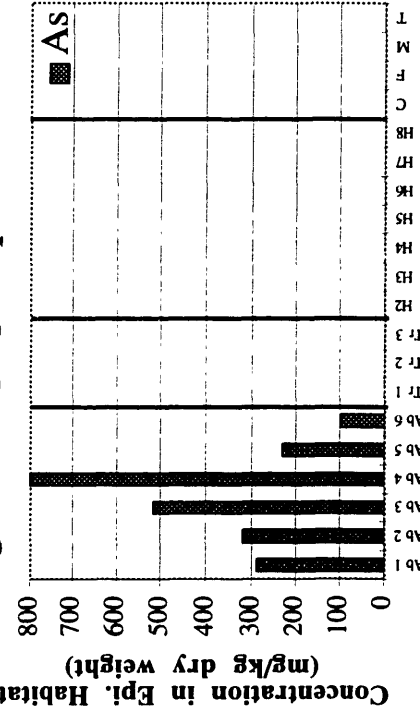


Fig. 2.5.b.2. [Cr] in Epifaunal Habitat

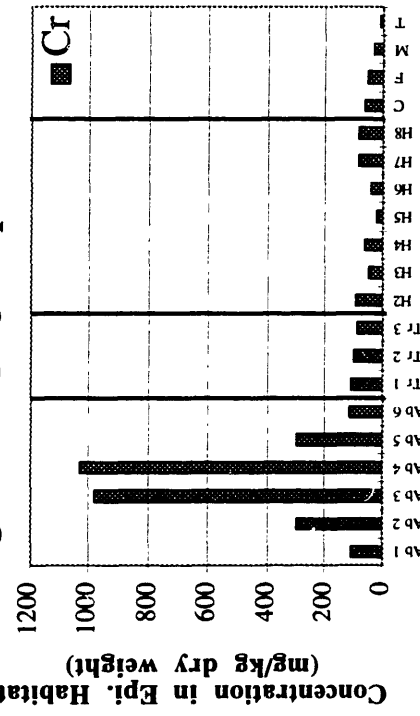


Figure 2.5 Concentrations of As, Cr, Cu, Pb and Zn, Cont'd

Fig. 2.5.a.3. [Cu] in Sediments

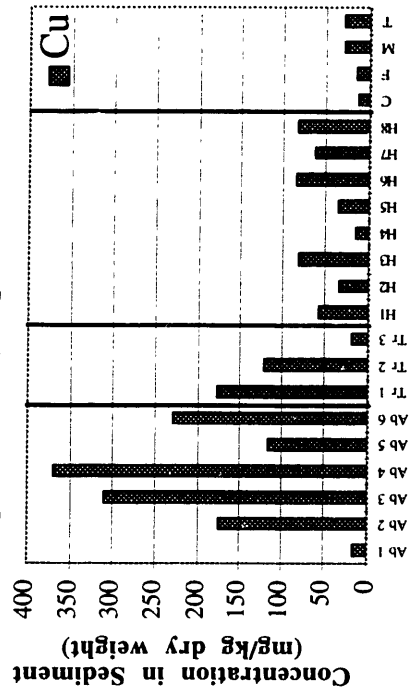


Fig. 2.5.a.4. [Pb] in Sediments

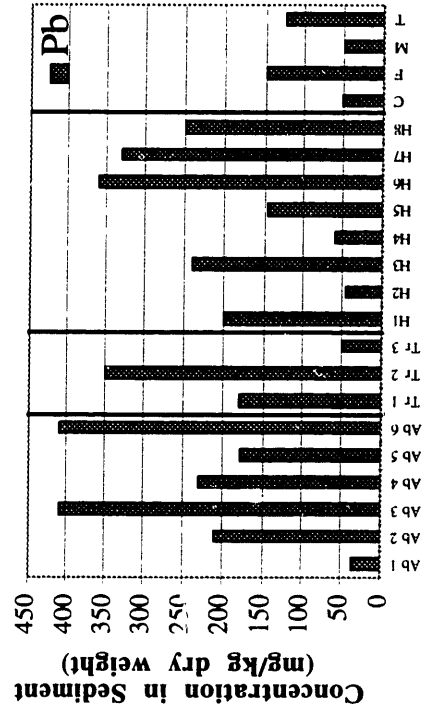


Fig. 2.5.b.3. [Cu] in Epifaunal Habitat

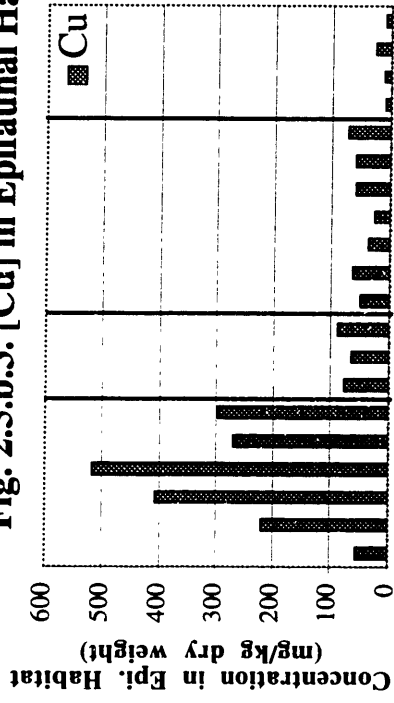


Fig. 2.5.b.4. [Pb] in Epifaunal Habitat

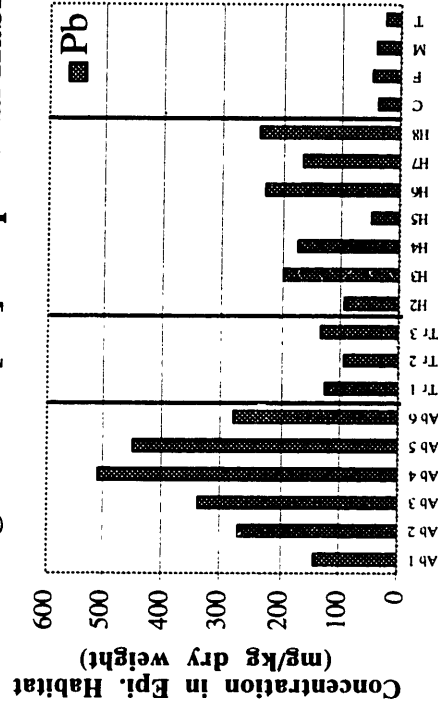


Figure 2.5 Concentrations of As, Cr, Cu, Pb and Zn, Cont'd

Fig. 2.5.a.5. [Zn] in Sediments

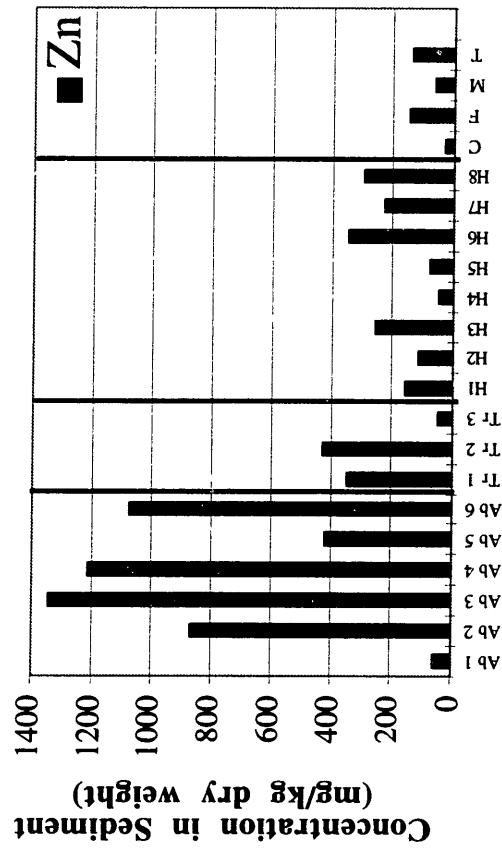
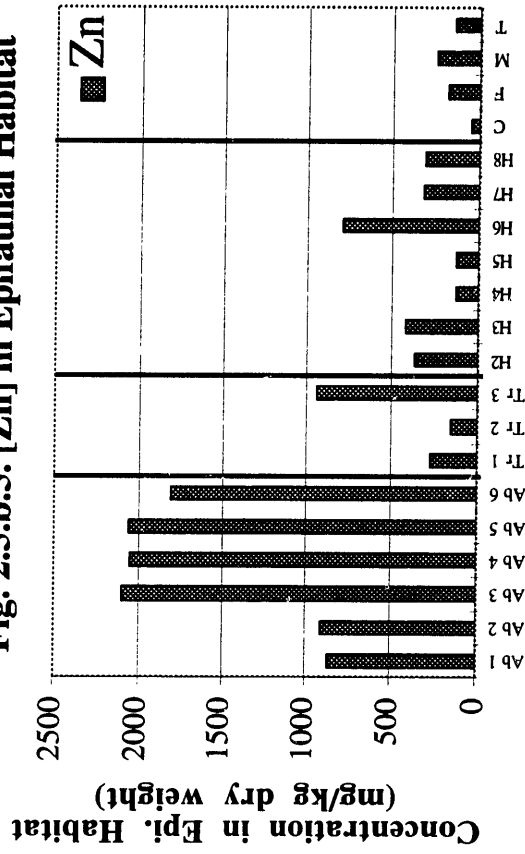


Fig. 2.5.b.5. [Zn] in Epifaunal Habitat



The concentrations of As, Cr, Cu, Pb and Zn did not seem to be strongly related to habitat sample type. Undercut bank roots, submerged vegetation, watercress, and overhanging bank vegetation had high concentrations of most metals at Aberjona River sites and low concentrations at other sites. Both undercut bank roots and scraped rocks were measured at site H3. Both had As concentrations below detection limits. Concentrations of Pb, Cu and Zn were about twice as high in the undercut bank root sample than in the scraped rock sample, while the concentration of Cu was twice as high in the root sample. Two samples of watercress, one heavily silted and one less silty sample, were measured at site H4. Concentrations of all elements were similar (As: <20, heavily silted sample, <20 less silty sample; Pb: 157, 174; Cr: 58, 60; Cu: 39, 36; Zn: 124, 127).

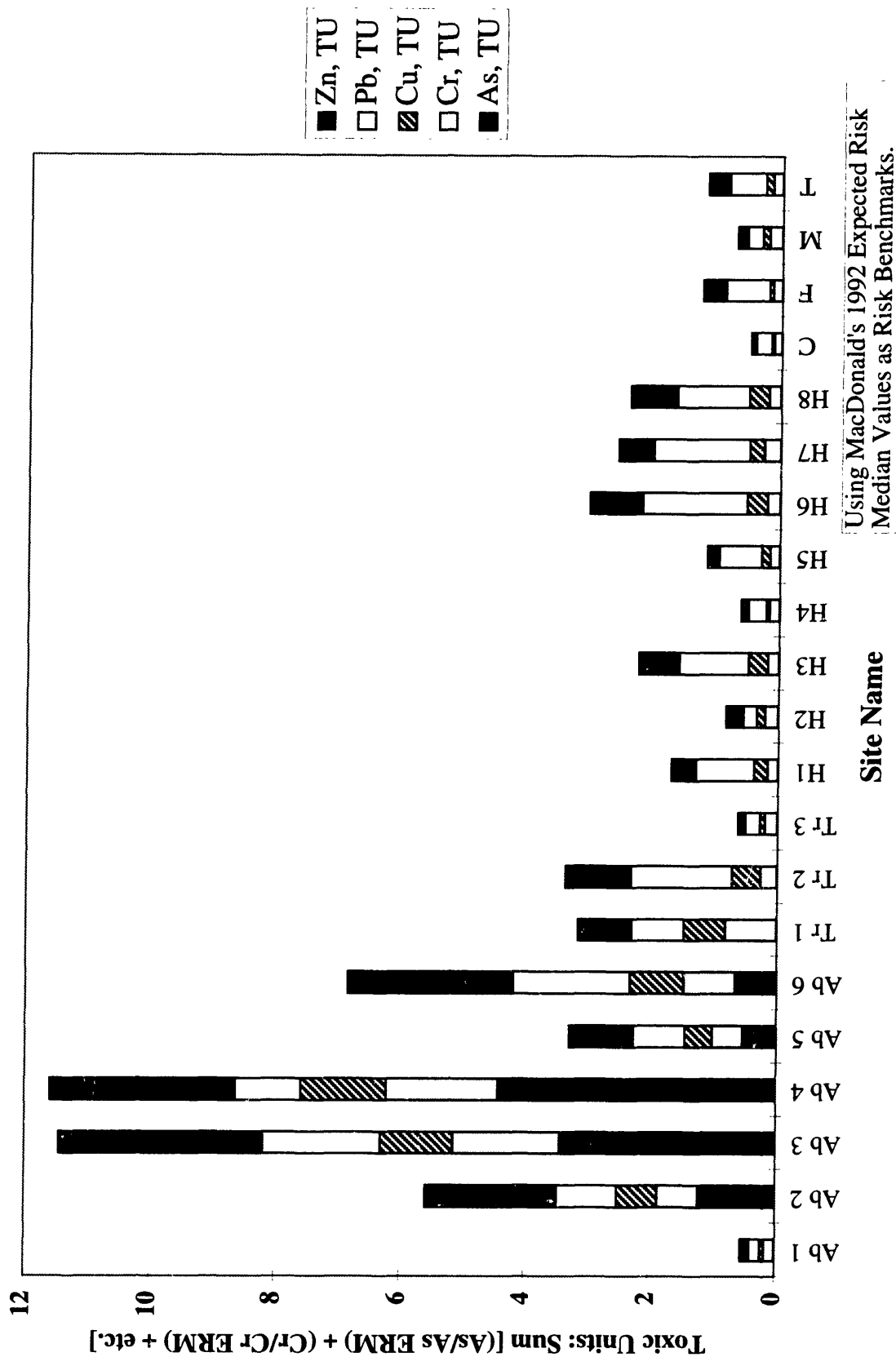
Concentrations of As, Cr, Cu, Pb and Zn in sediment and habitat samples were divided by ERL and ERM values (Long and Morgan 1990, updated by MacDonald 1992) to calculate risk ratios (expressed in dimensionless toxic units). Risk ratios in excess of 1 (indicating that the concentration in the sample was higher than the benchmark concentration) occurred for many elements and many sites (Fig. 2.6). The greatest risk ratios were calculated for habitat samples from the Aberjona River sites.

Discussion

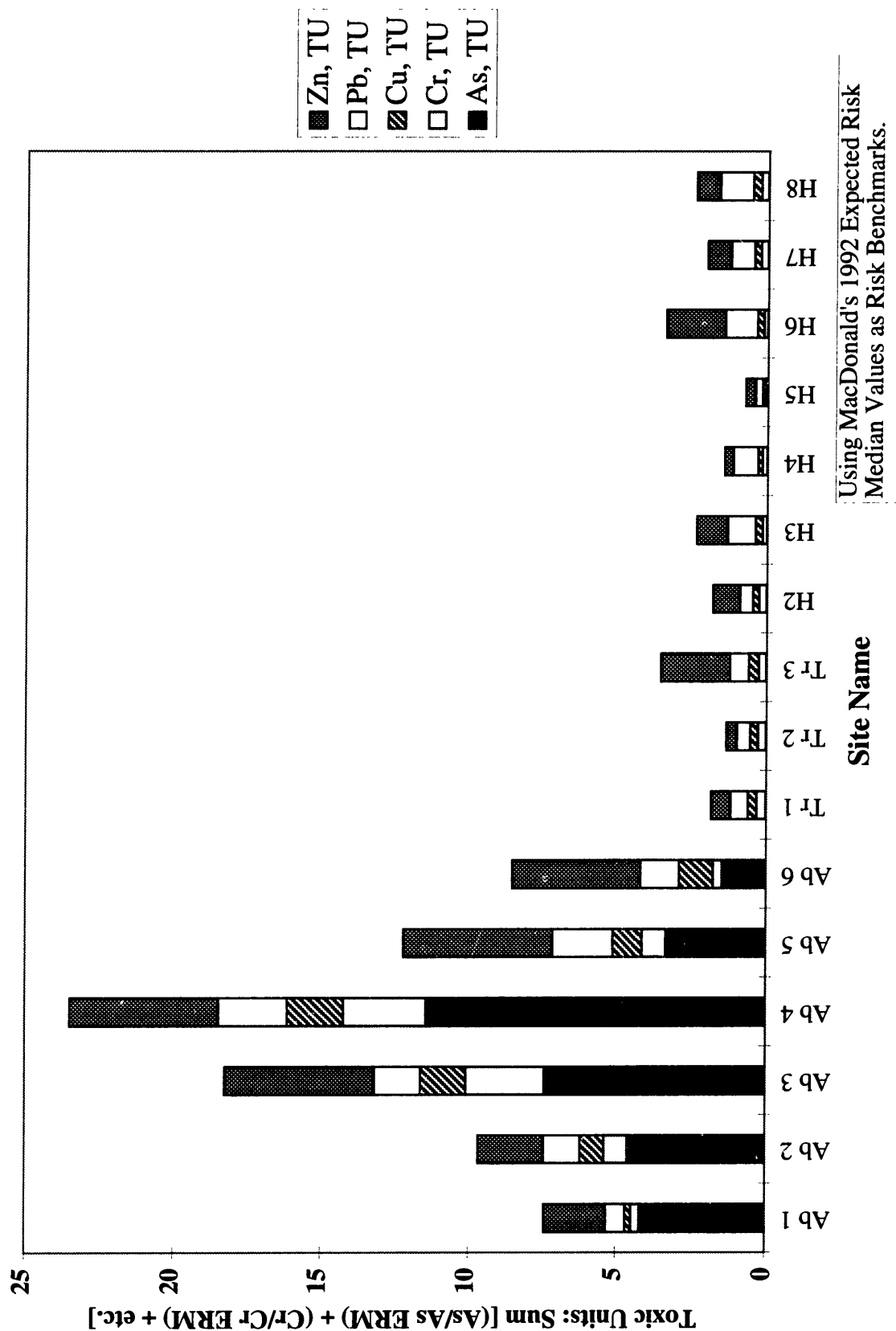
Physical Habitat Impairment

Urbanization has impaired the physical integrity of the Aberjona watershed in a number of ways (Table 2.3), and has resulted in decreased habitat for fish and macroinvertebrates (Fig. 2.3a). As the land has been developed for residential, commercial, and industrial uses, stream channels and the land beside them (the riparian zone) have been altered. Stream channels have been straightened, deepened, or diverted into artificial (e.g., concrete or metal) channels (Fig. 2.3c). Channel alterations have reduced sinuosity, and therefore have reduced the ability of streams to withstand storm surges (Tetra Tech 1995).

Figure 2.6.a. As, Cr, Cu, Pb, Zn Sediment Contamination
Toxic Units vs. Sites



**Figure 2.6.b. As, Cr, Cu, Pb, Zn Epifaunal Habitat Contamination
Toxic Units vs. Sites**



Stream channelization has reduced the variety of velocity depth combinations, resulting in a reduction of natural habitat for fish, macroinvertebrates and plants (Tetra Tech 1995).

Reference sites had optimal channel flow, while most Aberjona watershed sites had marginal or suboptimal flow (Fig. 2.3c). The low channel flow status at many Aberjona watershed sites was probably caused by disturbance of the natural hydrologic cycle. Rain falling on a vegetated surface is absorbed into the ground and slowly released to streams. In contrast, much of the rainfall in the Aberjona watershed falls on paved surfaces (such as buildings, roads and parking lots), which probably results in sharp increases in stream flow during storms and decreases in stream flow during dry periods. Residents have reported (personal communication) that flooding at sites Tr 2 and Tr 3 has at times increased stream depths to several meters (stream depths in August 1996 were less than 20 cm). Pollutants in urban runoff during such events may have impaired water quality, and the physical changes in the habitat that resulted may have adversely affected fish and macroinvertebrate assemblages. Flooding has also occurred on a less dramatic scale at many other locations. The impacts upon fish and macroinvertebrates of reduced baseflow may have been more severe than the effects of flooding. Low water levels probably decreased the availability of fish and macroinvertebrate habitat during dry periods, especially when the water level dropped below the level of overhanging bank vegetation and undercut bank roots and exposed these habitats to the air. The impairment of channel flow status at site H8 was caused by diversion of water by pumping from aquifers upstream, in the Horn Pond area. Although site H8 drains the entire Horn Pond subwatershed, it had a depth of only 20 cm in August 1996, and was completely dry in some sections in August 1997.

The relatively undisturbed riparian zones of the reference streams had abundant vegetation in wide strips along streams (Fig. 2.3d). Fallen trees, logs and branches provided habitat for fish and macroinvertebrates. Living bank vegetation also provided habitat in the form of undercut bank roots and overhanging bank vegetation (e.g., portions of shrubs leaning into the water). Fallen leaves provided food. Root systems held soil in

place; plants took up nutrients; trees provided shade. In contrast, in the Aberjona watershed, parking lots, roads, lawns and buildings have encroached upon the riparian zone, resulting in low scores for riparian vegetative zone width, bank vegetative protection and to a lesser extent, bank stability. In some areas, sediment deposition from eroding banks and other human disturbances covered logs and rocks, making them unavailable to macroinvertebrates and fish (Fig. 2.3b).

The difficulty we encountered in finding suitable reference sites suggests that habitat impairment is extremely widespread in the Boston Basin ecoregion. Examination of topographic maps (US Geological Survey topographic maps: Townsend, Ayer, Hudson, Billerica, Maynard, Framingham, Medfield, Franklin, Lawrence, Reading, Boston North, and Ipswich) and discussions with knowledgeable resource managers and scientists yielded only 50 sites in the ecoregion worthy of consideration; of these, only 4 sites were actually “minimally-impaired”. Of the fifty sites visited, U.S.G.S. maps indicated that uplands and riparian zones for several of these sites were relatively undeveloped with very few houses or other buildings, yet field visits revealed new housing developments in a large number of these areas. Apparently, in the years between the time when the aerial photographs were taken and the maps were checked in the field by the U.S.G.S. (1978-1982) and August 1996 when we investigated the sites, habitat quality has degraded considerably. Our set of candidate sites was limited to locations within walking distance of a road. Nevertheless, based on our experience, we believe that the problem of habitat degradation is increasing over time. Sites C and H7 are located in conservation areas, and are likely to retain their high habitat quality. Sites F, M and T, in contrast, are vulnerable to future degradation. This raises the concerns that these sites will be less suitable for use as “minimally-impaired” reference sites in the future and that areas of high quality habitat are disappearing, which implies that areas with biological integrity are also disappearing.

Sediment and Epifaunal Habitat Contamination by As, Cr, Cu, Pb and Zn

Concentrations of As, Cr, Cu, Pb and Zn in sediments from sites in the Aberjona watershed (Fig. 2.6) exceeded Long and Morgan's (1990, updated by MacDonald 1992) Expected Risk Low (ERL) and Expected Risk Median (ERM) values at many locations. Long and Morgan's criteria were developed for use by the National Status and Trends Program (NSTP) of the National Oceanic and Atmospheric Administration (NOAA) (Long and Morgan 1990, MacDonald 1992, Long and MacDonald 1992). The NSTP Approach presumes that the potential for toxicity is relatively high in areas where numerous chemicals exceed the ERM, and that the potential for toxicity is low in areas where none of the chemical concentrations exceed the ERL.

This assumption limits the accuracy of the method because many factors mediate whether or not the presence of high concentrations of contaminants cause biological impairment. Acid volatile sulfide binds some metals, reducing their toxicity. Organic carbon can also prevent toxic exposures. Aspects of water chemistry, such as pH and hardness, also affect the toxicity of metals. In addition, contaminants only affect organisms if they are exposed and sensitive to that stressor. An organism's response to a mixture of contaminants is not necessarily equal to the sum of the organism's response to individual contaminants. Finally, it is probably also true that organisms respond differently to toxic metals in physically degraded systems than they do in optimal physical habitats, but this is not well studied. The guidelines do not yet account for the effects of factors that control bioavailability of toxicants; if the necessary data become available, then ERL and ERM concentrations may be normalized (e.g., to account for acid volatile sulfide and total organic carbon concentrations and other potential normalizers) (Long and MacDonald 1992).

NOAA used the NSTP approach to identify chemicals that occurred in concentrations that were sufficiently high to warrant concern, to identify sampling sites and areas in which there was a potential for toxicity, and to assess and prioritize hazardous

waste sites (Long and MacDonald 1992). The NSTP approach has also been used by Environment Canada and the California Water Resources Control Board in their development of sediment quality guidelines (Long and MacDonald 1992). Recommended uses of the guidelines (Long and MacDonald 1992) include ranking and prioritization of areas and sampling sites for further investigation, quantification of the relative likelihood of toxicity over specific ranges of chemical concentrations, and assessment of potential ecological hazards of contaminated sediments.

Concentrations of Cr, Cu and Zn in the sediments of Horn Pond subwatershed and Tributary sites frequently exceeded ERL but not ERM values, indicating the possibility of adverse effects (Fig. 2.5, 2.6). Arsenic concentrations at these sites were uniformly below the detection limit of about 20 ppm. The ERL for As is 8.2 ppm, thus the possibility of adverse effects due to As at these sites cannot be eliminated. Concentrations of Pb in sediments of the Horn Pond subwatershed and Tributary sites usually exceeded the ERL value, and sometimes exceeded the ERM value, indicating the likelihood of adverse effects.

Concentrations of As, Cr, Cu and Zn were significantly higher in the sediments of many Aberjona River sites than in the sediments of other sites. Correspondingly, the exceedances (of ERL and ERM) are much greater. Thus, the NSTP approach indicates that the greatest ecological hazard existed at sites Ab 3 and Ab 4, where exceedances were greatest, and that great hazard also existed at sites Ab 2, Ab 5 and Ab 6.

The NSTP approach was developed for sediments. Many benthic organisms have greater exposure to undercut bank roots, overhanging bank vegetation, and vegetation attached to rocks than to fine-grained sediments. It is sometimes assumed that the highest concentrations of contaminants occur in fine-grained (especially organic rich) sediments. While these concentrations usually exceed the concentrations found in coarser sediments (confirmed in the Aberjona watershed for sandy sediments), interestingly, the concentrations of As, Cr, Cu, Pb and Zn were frequently higher in epifaunal habitat samples than in fine-grained sediments from the same site. Although the NSTP approach

was not developed for epifaunal habitat samples, it may be ecologically significant that the concentrations of these elements in epifaunal habitat samples greatly exceed ERL and ERM values for sediments at sites Ab 1, Ab 2, Ab 3, Ab 4, Ab 5 and Ab 6. The difference in concentrations, and thus toxic ratios (toxic ratio = concentration / ERM), between Aberjona River sites and Horn Pond and Tributary sites was much greater in the epifaunal habitat samples than in the sediment samples.

Table 2.4. Metals: Discussion of risk

<u>Metal</u>	<u>Discussion</u>
As	Sites Ab 2-6 were probably impacted by As. Site Ab 1 had high concentrations of As in the epifaunal habitat sample, but not in the sediment sample.
Cr	Sites Ab 3 and 4 were probably impacted by Cr, other sites may also have been adversely impacted.
Cu	Biological assemblages may have been adversely affected by Cu, but the risk was probably less significant than the risk for As and Cr given that concentrations were rarely above the ERM and these concentrations were less than two times the ERM at the most contaminated sites.
Pb	The impacts of Pb were probably more widespread but less severe than the impacts of As and Cr.
Zn	<p>Zn concentrations may have been higher in habitat samples than in sediments because Zn is an essential nutrient for plants. Zn exceedances at H3 and Tr 2 were small, and only occurred in habitat samples. Larger exceedances occurred at sites H6 and Tr 3.</p> <p>The high Zn concentrations found in the most contaminated habitat samples greatly exceeded the concentrations of Zn in the reference samples. The highest Zn concentration in any reference site habitat sample is 250, which is well below the ERM of 410. Further study would be needed to fully elucidate the risk of high concentrations of Zn in epifaunal habitat samples.</p>

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Chapter 3:

Impacts of Metal Contamination and Physical Habitat Degradation upon Fish Assemblages of the Aberjona Watershed, MA.

C.E. Rogers, A. Peter, M.T. Barbour, and H.F. Hemond

Abstract. The cumulative impacts of physical habitat degradation and metal contamination of sediments upon fish assemblages of an urban watershed in Massachusetts (the Aberjona River watershed) were evaluated. Fish populations were sampled at 16 locations by wading method electroshocking. The biological condition of fish assemblages was characterized and related to habitat condition and chemical contamination. As predicted, in the absence of contamination, biological condition varied from good to poor as habitat condition varied from good to poor. Also as predicted, biological degradation beyond that attributable to habitat degradation alone was associated with contamination of sediments and epifaunal habitats by As, Cr, Cu, Pb and Zn. The predicted relationship was observed when: (a) the number of native fish species caught at a site was used as the measure of biological condition, (b) habitat condition was a simple function of instream cover score (a visually estimated habitat parameter: the percentage of the sampling site with a mix of snags, submerged logs, undercut banks, rubble, or other stable habitat) and stream depth, and (c) a site was considered contaminated if concentrations of As, Cr, Cu, Pb and/or Zn exceeded Expected Risk Median values (MacDonald 1992). No fish were caught at 6 sites with stream depths of less than 20 cm; the maximum number of species caught at a site was 8 native and 2 non-native species. Urbanization of the Aberjona watershed appears to have contributed to decreases in stream depth and instream cover for fish. Contamination of stream sediments and epifaunal habitat by As and Cr was reflected in elevated concentrations in whole ground white suckers. Concentrations of Zn in whole ground fish were not related to sediment and epifaunal habitat contamination.

Introduction

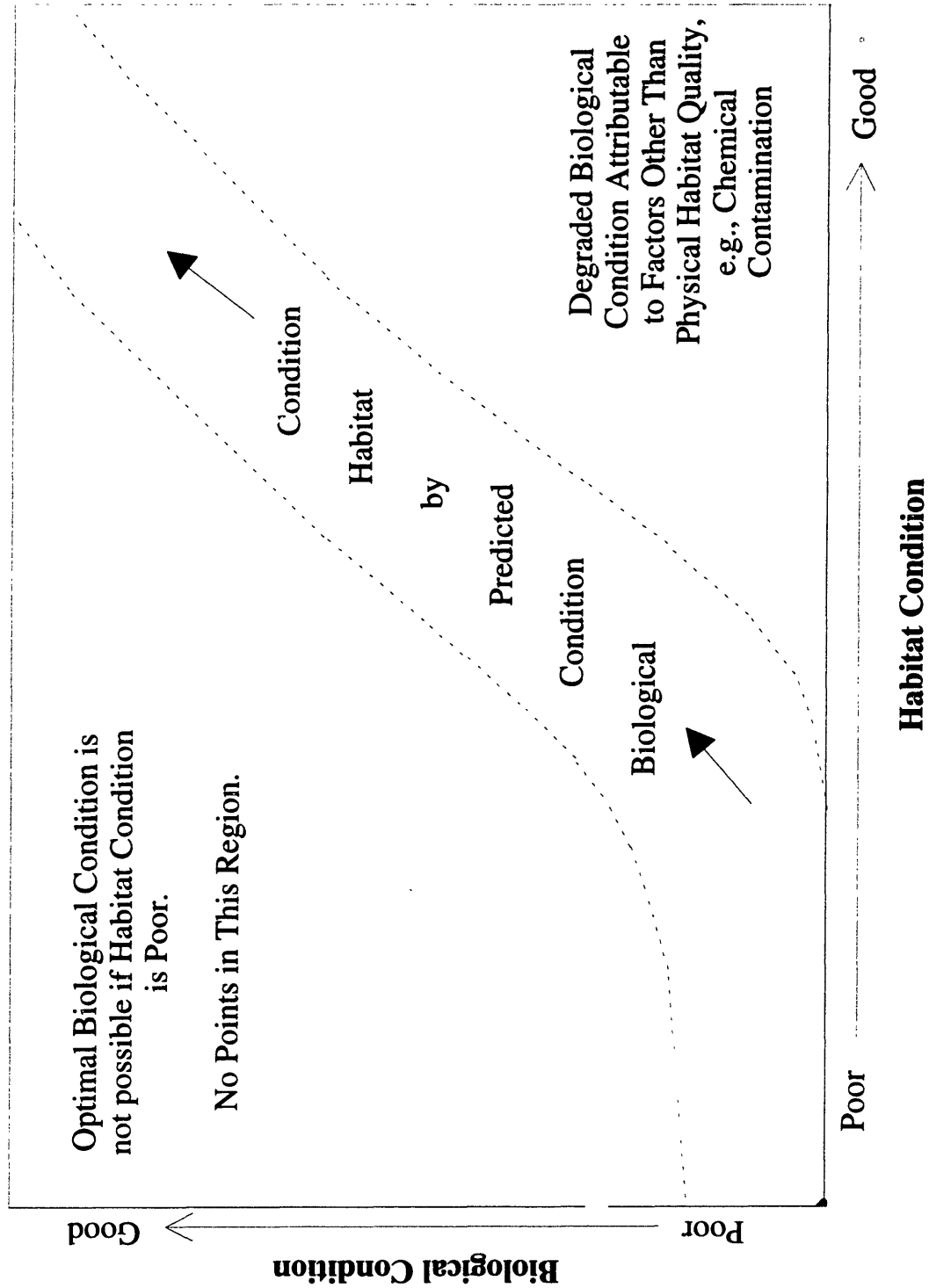
The biological integrity of freshwater fish assemblages is of concern to the public because of the direct (cultural, religious, recreational, economic, and aesthetic) value placed upon fish, and because fish assemblages reflect overall ecological integrity. Fish account for nearly half of the endangered vertebrate species and subspecies in the United States (Barbour *et al.* 1997). Some argue that top-level (piscivorous) predatory fish are useful as indicator species because to support a top-level predator, an ecosystem must also provide appropriate habitat for lower trophic levels (Imhof *et al.* 1996, p. 321 citing Bowlby and Roff 1986b, Northcote 1988, Perrin *et al.* 1987, Power 1990, 1992, Power *et al.* 1995). Fish are good indicators of long-term effects and habitat conditions (Karr *et al.* 1986); the

characteristics of fish assemblages reflect the influences of chemical and physical stressors and provide a broad measure of the aggregate impact of stressors (Barbour *et al.* 1997).

Previous studies have evaluated the impacts of physical habitat degradation or chemical contamination upon fish assemblages (e.g., Birge *et al.* 1977, Gorman & Karr 1978, Griswold *et al.* 1978, Schlosser 1982, Francis *et al.* 1984, Herbold 1984, Frissel *et al.* 1986, Dallinger *et al.* 1987, Sprague 1987, Dawson *et al.* 1988, Mac 1988, Steedman 1988, Bendell-Young & Harvey 1989, Schlosser 1990, Schaefer & Brown 1991, Bryan & Langston 1992, Pascoe & DalSoglio 1994, Weaver & Garman 1994, Ostrander *et al.* 1995, Rankin 1995), but integrated assessment of both types of impacts is needed. A graphical framework for relating biological condition to physical habitat degradation and chemical contamination was proposed by Barbour and Stribling (1991) and further elaborated by Barbour *et al.* (1996a, 1997); their framework is represented in Figure 3.1. Once measures of biological condition and habitat condition have been selected, the framework can be used to test the predictions that: (a) in the absence of chemical contamination, biological condition varies from good to poor as habitat condition varies from good to poor, and that (b) degraded biological condition in the absence of physical habitat degradation is attributable to something (e.g., chemical stressors) other than habitat quality (Barbour *et al.* 1996a, 1997).

Detecting impacts upon fish assemblages and identifying their causes is a great challenge for environmental managers because limited resources are available for data collection and interpretation. Multimetric indices are frequently used by state environmental protection agencies and the U.S. Environmental Protection Agency (U.S. EPA, Davis *et al.* 1996) to detect aquatic biological impairment, to measure physical habitat degradation, and to compare concentrations of contaminants to levels associated with adverse biological effects (e.g., Fausch *et al.* 1984, Karr *et al.* 1986, Miller *et al.* 1986, Ohio EPA 1987, Long & MacDonald 1992, Rankin 1995, Tetra Tech 1995, Barbour *et al.* 1997). Greater integration of the results of these chemical, physical and biological assessments would facilitate the identification of the chemical and physical causes of biological impairment.

**Figure 3.1 Conceptual Model of Barbour *et al.* (1997)
Relationship of Biological Condition to Habitat Condition**



The purpose of the present investigation was to evaluate the cumulative impacts of physical habitat degradation and metal contamination of sediments upon fish assemblages of an urban watershed in Massachusetts (the Aberjona River watershed). The fish of the Aberjona watershed (which is located 12 miles north of Boston) have been subjected to a variety of chemical and physical disturbances. Most notably, the Aberjona River's chemical integrity has been compromised by two U.S. EPA National Priority Listed Superfund sites; concentrations of As, Cr, Cu, Pb and Zn in Aberjona River sediments exceed Long and Morgan's Expected Risk Median values (Long and Morgan 1990, MacDonald 1992, Long and MacDonald 1992) in many locations (chapter 2). Development of land within the watershed for residential, commercial and industrial purposes has compromised physical integrity. Physical and chemical disturbances were characterized with multimetric indices (developed by Tetra Tech 1995 and Long and MacDonald 1992), and the concentrations of As, Cr and Zn in fish were measured. The biological condition of fish assemblages was characterized with metrics recommended for use in New England (Miller *et al.* 1986). Application of the Barbour *et al.* (1997) graphical framework to the Aberjona watershed required the selection of appropriate measures of habitat condition and biological condition. We hypothesized that the most important components of habitat condition would be total habitat score (the total score for the multimetric index of physical habitat degradation), availability of instream cover score (the score for one metric of the multimetric index), and stream depth.

Methods

Fish Sampling

Seventeen locations were selected in the Aberjona Watershed (Fig. 1.a) and fish populations were sampled (September 30-October 9, 1995) at each site by wading method electroshocking using a Coffelt CPS-system DC pulsed electroshocker. To estimate population densities, a removal method was performed using two runs (White *et al.* 1992).

In cases where the catch was very low (only a few individuals), one single run was carried out. After anaesthetizing the fish (MS 222), each was identified to the species level, its fork length was measured and obvious signs of gross disease were recorded. Some fish were taken back to the laboratory for metal analysis. The remainder were allowed to recover in stream water and were put back into the stream at the location where they were caught.

Electroshocking was successful at 16 locations. At one location (Ab 6), the water was too deep and turbid for effective collection. Of the 16 successfully fished locations, 5 sites are located on the Aberjona River, 3 sites are located on small tributaries to the Aberjona River (Halls Brook, North Woburn Creek, and Sweetwater Brook), and 8 are located in the Horn Pond subwatershed. Fish were caught at 10 locations and no fish were observed at 6 locations.

Selection of a Minimally-Impaired Site

One stream reach (site H7) within the watershed has suffered relatively little chemical or physical disturbance, and is located within a wooded parcel of conservation land. Chemical and physical impairment of this stream reach was compared to the best “natural” habitat that could be found within the ecoregion (the Boston Basin Ecoregion, Griffith *et al.* 1994). The chemical and physical parameters of site H7 were similar to those of these reference sites. It is likely that the fish assemblage of this relatively well-protected reach was impacted by disturbances in other parts of the watershed, but biological integrity is probably higher at this location than at other locations within the Aberjona watershed. Thus, this site was chosen to represent least-impaired conditions for the watershed.

Characterization of the Biological Condition of Fish Assemblages

A multimetric approach is recommended to characterize the biological condition of freshwater streams to provide detection capabilities over a broad range of stressors

(Gammon 1980, Karr 1981, Fausch *et al.* 1984, Karr *et al.* 1986, Miller *et al.* 1986, Ohio EPA 1987, Steedman 1988, Plafkin *et al.* 1989, Karr 1991, Barbour *et al.* 1996b, Gibson *et al.* 1996, Barbour *et al.* 1997). The index of biotic integrity (IBI) is one widely used multimetric approach (e.g., Miller *et al.* 1988); the index requires adaptation to regional conditions (Karr *et al.* 1986, Miller *et al.* 1986, Miller *et al.* 1988, Steedman 1988). Miller *et al.* (1986) adapted Karr's (1981) index of biotic integrity (IBI) for use in the Merrimac and Connecticut River Drainages in Massachusetts and New Hampshire. The following 12 metrics were adopted: 1) Total number of fish species, 2) Number and identity of native water column species, 3) Number and identity of native benthic insectivorous species (excludes insectivorous sucker species), 4) Number and identity of native sucker species, 5) Number and identity of native intolerant species, 6) Proportion of individuals as white sucker, 7) Proportion of individuals as omnivores (excludes white sucker), 8) Proportion of individuals as insectivores, 9) Proportion of individuals as top carnivores, 10) Density of individuals in sample, 11) Proportion of individuals as hybrids, and 12) Proportion of individuals with disease, tumors, fin damage or other anomalies.

The aggregation of metrics into a multimetric index (e.g., the index of biotic integrity) is possible if each metric can be scored according to the value observed or expected in a minimally-disturbed stream of similar size in the same region (e.g., Steedman 1988); the aggregate index is the sum of the scores for each metric. Site H7 was not expected to adequately represent minimally-disturbed conditions because: (a) the fish assemblage of this relatively well-protected reach were impacted by disturbances in other parts of the watershed, (b) the fish assemblage at site H7 may be affected by its proximity to a large pond (Horn Pond), and (c) a set of ecoregional reference sites is preferable for establishing a reference condition; one site cannot normally capture natural variability. Thus, metric values at site H7 were not used to score metrics for aggregation into an index.

Another approach to metric scoring in widely degraded systems is to calculate the 95th percentile value of metrics that decrease in response to perturbation, and the 5th

percentile value of metrics that increase in response to perturbation. Then the region from 0 to the 95th percentile value (or 5th percentile to maximum value) can be trisected and regions can be scored 1, 3, or 5, with high quality sites receiving the highest scores. (A similar scoring concept is used to construct the Index of Biotic Integrity [e.g. Karr *et al.* 1986, Karr 1991].) This approach was also rejected for the Aberjona watershed because of the 10 sites where fish were caught, only site H7 approaches a reference condition. Thus, the 95th percentile (or 5th percentile) value of each metric would not necessarily have represented the optimal value.

Thus, the 12 metrics recommended by Miller *et al.* (1986) were calculated and their values at sites with varying degrees of physical and chemical disturbance were compared to the values calculated for each metric at the less-disturbed site H7, but an aggregate index of biological condition was not constructed for this paper.

Habitat Parameters

Physical habitat assessment was performed at each site (August 1996) according to the protocols used by the Massachusetts Department of Environmental Protection (Tetra Tech 1995) modified for slow-flow, low gradient streams. Ten habitat parameters (instream cover, epifaunal substrate, embeddedness, channel alteration, sediment deposition, variety of velocity-depth combinations, channel flow status, bank vegetative protection, bank stability, and riparian vegetative zone width) were scored from 0 to 20 (Appendix A). Instream cover was evaluated according to the percentage of the sampling site with a mix of snags, submerged logs, undercut banks, rubble, or other stable habitat.

At the time of fish sampling (October 1995), measurements of stream depth and width were taken at 10m intervals for each stream reach sampled except for sites H1, Ab 3 and Ab 4. Physical habitat assessment performed the following August (1996) included one measurement of stream depth that was visually estimated to be representative of the stream reach's depth. The average value of the stream depth measurements made in

October 1995 were similar in value to representative measurements made in August 1996 (Appendix B).

Elemental Analysis of Sediment and Epifaunal Habitat

Stream sediments and epifaunal habitat samples (i.e., submerged vegetation, vegetation-covered rocks, overhanging bank vegetation, and undercut bank roots) were collected at all sites (method: chapter 2).

Five (5.0) grams of each dried sample were pulverized in a Spex Mixer cartridge with a silicon carbide ball for 5 minutes. One-half gram (0.5 g) of copolywax (TM) binder was added to the sample and mixed again for 1 minute. This mixture was poured into a 31mm diameter aluminum sample cup and pressed for 1 minute using 12 metric tons of force in an evacuable die. Elemental concentrations in pellets were analyzed by X-Ray Fluorescence (XRF) using a Philips PW1480 wavelength dispersive XRF.

Concentrations of As, Cr, Cu, Pb and Zn were compared to sediment benchmark values that have been associated with adverse biological effects (Long and Morgan 1990, MacDonald 1992, Long and MacDonald 1992):

Table 3.1. Expected Risk - Low (ERL), and Expected Risk - Median (ERM) values for sediments (MacDonald 1992)

	As	Cr	Cu	Pb	Zn
ERM (ppm) (dry weight)	70	370	270	223	410
ERL (ppm) (dry weight)	8.2	81	34	46.7	150

Results of contamination analysis are presented as graphs (Fig. 3.6) of toxic units. The concentration of an element (e.g., As) in a (sediment or epifaunal habitat) sample was normalized by the benchmark (e.g., ERM: 70 ppm) to calculate the toxic units of that element at a site. Individual elements are graphed using stacked bars for each site, thus

displaying the individual toxic units contributed by each element as well as the sum of all toxic units for a site.

Characterizing the Relationship of Biological Condition to Habitat Condition and Chemical Contamination

Application of the Barbour *et al.* (1997) graphical framework (Fig. 3.1) to the Aberjona watershed required the selection of appropriate measures of habitat condition and biological condition. An aggregate (multimetric) index should be a better measure of biological condition than any individual benthic metric because an aggregate index encompasses several aspects of the structure and function of the community (Barbour *et al.* 1996b). However, for reasons discussed above, an aggregate index could not be appropriately developed for this study. The number of species caught at a site (Fig. 3.2) was chosen as a surrogate measure of biological condition. The rationale for this choice is explained in the results section.

We hypothesized that the most important components of habitat condition would be total habitat score (the total score for the multimetric index of physical habitat degradation), availability of instream cover score (the score for a metric of the multimetric index), and stream depth. The instream cover score is a component of total habitat score, thus only one of these two measures was included in a habitat condition index. The relationship of biological condition to instream cover score was stronger than the relationship of biological condition to total habitat score (Fig. 3.4, 3.5). An empirical value of 20 cm was observed as a threshold for stream depth below which fish were generally not caught. Thus an index of habitat condition was constructed that was: (a) normalized to range from 0 to 1, (b) equal to 0 if stream depth was below the threshold value of 20 cm, and (c) that was evenly weighted between stream depth and instream cover score for stream depth values above 20 cm:

HABITAT CONDITION INDEX

Habitat Condition = 0 if stream depth < 20.
= 0.5 [(SD/ max SD) + (IC /max IC)]

where

SD = stream depth

max SD = max stream depth of all sites (= 62 cm)

IC = instream cover score

max IC = maximum instream cover score of all sites (= 18)

The relationship of biological condition to overall habitat quality, the availability of instream cover, and stream depth was evaluated graphically. The relationship of biological condition to habitat condition and chemical contamination was evaluated using the Barbour *et al.* (1997) graphical framework (Fig. 3.1): the number of species caught at a site was plotted versus the habitat condition index. The following predictions were tested: (a) that in the absence of chemical contamination (reflected by low total toxic unit scores), biological condition would vary from good to poor as habitat condition varied from good to poor, and that (b) biological degradation beyond that predicted by habitat condition could be explained by contamination (reflected by high total toxic units scores).

Analysis of Metal Concentrations in Fish

Fish retained for metal analysis were stored frozen in polyethylene bags until March 1996 when they were thawed, processed, and analyzed for metals by instrumental neutron activation. In the process of freezing and thawing, a small amount of liquid was released from the fish and stuck to the polyethylene bags. As a result, the masses of the fish decreased slightly, usually less than 8-15%. Fish lengths were measured from the tip of the mouth to the tip of the tail (full length) and from the tip of the mouth to the fork of the tail (fork length). In cases where fish tails were torn and the fork could not be distinguished, only the full length was recorded. Using an Omni Mixer Homogenizer with a titanium cutting blade, fish were ground into a pulp inside acid-washed polyethylene bottles (with the caps cut off). Less than 0.2 g of sample was transferred into acid-washed sample bags with nickel forceps and put into vials for neutron activation analysis.

Approximately one gram of the homogenous fish mixture was transferred into pre-weighed aluminum tins and weighed before and after drying. (Dry weights were recorded until weight was constant.) Samples were dried in a 100°C oven at least overnight.

The samples were irradiated inside the Massachusetts Institute of Technology (MIT) nuclear reactor with a thermal neutron flux of $8 \times 10^{12} \text{ n cm}^{-2} \text{ sec}^{-1}$ for 12 hours. The samples were then cooled for 2-3 days and transferred to clean polyethylene bags for counting. Gamma ray emissions were measured with high purity germanium detectors coupled to 8192-channel pulse-height analyzers (Canberra, CT). To detect the gamma peaks of each isotope, the spectra were analyzed using the computer program ND 9900 Genie run on a VMS 200 computer (Canberra, CT). A fly ash standard reference material (SRM 1633), purchased from the National Institute Standards and Technology (NIST), was used to calculate elemental concentrations. Two other reference materials, SRM 1571 (orchard leaves) and SRM 1577a (bovine liver), were used to check the system stability. Bovine liver was put into polyethylene bottles, ground, and put in contact with the titanium blade and analyzed for quality control. All samples, standards and control samples were counted for 12 hours at a constant geometry. Additional details of the analytical procedure have been published elsewhere (Olmez 1989).

Concentrations of As, Cr and Zn in fish were also measured by the U.S. EPA (data supplied by Mary Garren, U.S. EPA Region 1, data collected as part of ongoing studies of the Wells G and H Superfund Site). Data from the EPA study is included in Figure 3.8.

Results

Biological Condition of Fish Assemblages

Table 3.2. Fish Metric Results

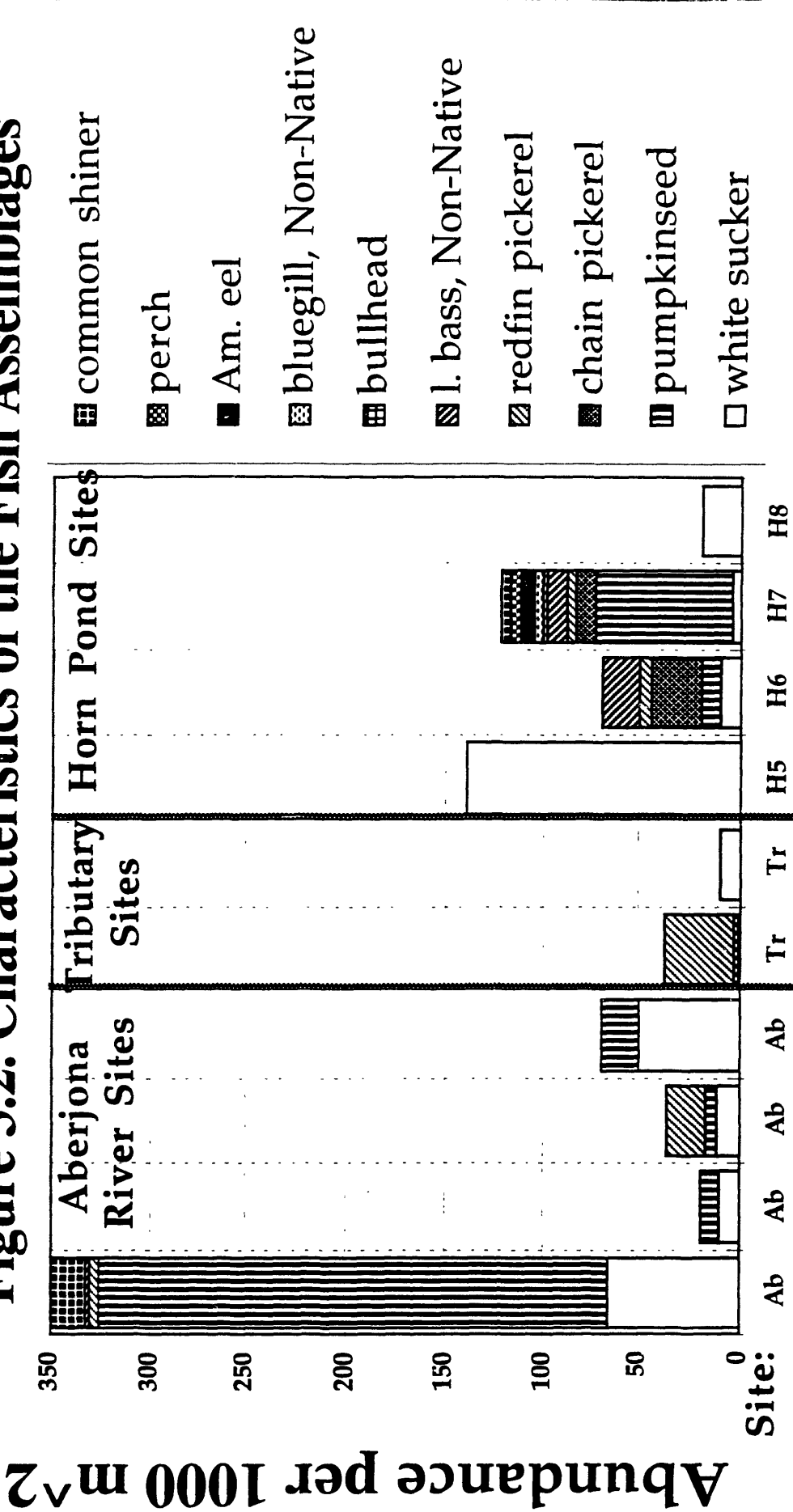
<u>Metrics Recommended by Miller <i>et al.</i> (1986)</u>	<u>Results</u>
1. Total number of fish species	8 native, 2 non-native species (bluegill and largemouth bass)
2. Number and identity of native water column species	pumpkinseed, redbfin pickerel, chain pickerel, common shiner, American eel, yellow perch
3. Number and identity of native benthic insectivorous species (excludes insectivorous sucker species)	brown bullhead, present only at site H7
4. Number and identity of native sucker species	white sucker, present at all sites
5. Number and identity of native intolerant species	none
6. Proportion of individuals as white sucker	These metrics were combined (% omnivores). Common shiner composes 7% of the omnivores at site Ab 2 and 2% at site H7. White sucker is the only other omnivore.
7. Proportion of individuals as omnivores (excludes white sucker)	
8. Proportion of individuals as insectivores	% insectivores
9. Proportion of individuals as top carnivores	% piscivores
10. Density of individuals in sample	abundance per 1000 sq. meters
11. Proportion of individuals as hybrids	none
12. Proportion of individuals with disease, tumors, fin damage or other anomalies	black spot disease was the only disease identified

16 sites were sampled by electroshocking; fish were caught at 10 locations. Electroshocking was attempted at a 17th site (site Ab 6, Fig. 1.a). Fish were observed in the water column of pond habitat close to this site, but the depth and turbidity of the water at site Ab 6 prevented effective sampling. Fish were caught at Aberjona River sites Ab 2-5, Horn Pond sites H5-H8, and Tributary sites Tr 1 and Tr 2 (Fig. 1.a).

The total number of species caught at each site ranged from 0 (6 sites) to 10 (1 site); the number of native species ranged from 0 to 8 (Fig. 3.2). White sucker (*Catostomus commersoni*) was caught at all ten sites where fish were caught. Pumpkinseed (*Lepomis gibbosus*) was caught at all sites with two or more species (7/10 sites). Redfin pickerel (*Esox americanus americanus*) was caught at all sites with three or more species (5/10 sites). Largemouth bass (*Micropterus salmoides*) was caught at all sites with four or more species (3/10 sites). Common shiner (*Notropis cornutus*), an omnivore, was caught at sites Ab 2 (5 species caught) and H7 (10 species caught). Chain pickerel (*Esox niger*) was caught at sites H6 (5 species caught) and H7. Yellow perch (*Perca flavescens*), American eel (*Anguilla rostrata*), bluegill (*Lepomis macrochirus*) and brown bullhead (*Ameiurus nebulosus*) were only caught at site H7. Bluegill (only caught at site H7) and largemouth bass (caught at sites Ab 2, H6 and H7) were the only non-native fish species caught in the Aberjona watershed (classification as native or non-native: Hartel, 1992).

The greatest abundance of fish was found at site Ab 2, which had 350 fish per 1000 square meters of stream surface area (Fig. 3.2). This site's high abundance reflects a large catch of young pumpkinseeds. Abundance was also high at site H5, which had an abundance of 140 fish per 1000 square meters. Abundance was lower at other sites, ranging from 10 to 70.

Figure 3.2. Characteristics of the Fish Assemblages



% Dom.	74%	50%	42%	76%	73%	100%	100%	100%	33%	56%	100%
Abun.	350	20	40	67	35	10	142	70	4+1N	120	20
# Species	4+1N	2	3	2	3	1	1	1	4+1N	8+2N	1

N= Non-Native Species, number of

The values for % dominance ranged from 33% (H6) to 100% (Tr 1, H5, and H8) (Fig. 3.2). Several sites were dominated by white sucker. Two sites (Ab 2, H7) were dominated by pumpkinseed, and Tr 1 was dominated by redbfin pickerel.

White sucker and common shiner were the only omnivorous species present in the watershed (Fig. 3.3). Sites Ab 5, Tr 2, H5, H8 were dominated by omnivores. Piscivorous fish were caught at sites Ab 2, Ab 4, Tr 1, H6, and H7. Insectivorous fish were caught at sites Ab 2, Ab 3, Ab 4, Ab 5, Tr 1, H6, and H7.

Fish showed only one sign of gross disease. This was identified as "black spot" disease, caused by infection by the diagenetic trematode *Neascus* (P.R. Bowser, Ph.D., November 1995, personal communication to Ken Munney of the US Fish and Wildlife Service). Fish at site Ab 5 were heavily infested with *Neascus*: most fish had black spots covering 30-100% of their bodies. Diseased fish were also found at site Ab 2.

Relationship of Biological Condition to Habitat Condition and Chemical Contamination

Two sites where five species were caught differ in composition by one species. In all other cases, sites with the same number of species also have the same set of species. When two sites are compared, the site at which a greater number of species was caught always has a composition equal to the site at which a smaller number of species was caught plus at least one additional species. Sites at which only one species was caught are entirely composed of benthic omnivores. Sites at which only two species were caught are composed of omnivores and insectivores. Sites where redbfin pickerel were caught are composed of omnivores, insectivores and piscivores. Thus, in this study, the number of species caught at a site was an unusually informative measure of biological condition, and was therefore selected as the measure of biological condition to be used in interpreting the relationship between biological condition and habitat condition and chemical contamination.

Figure 3.4 illustrates the relationship of the number of fish species caught at a site to the site's stream depth and instream cover score. Sites with stream depths of less than

Figure 3.3. Percentages of each feeding group at each site

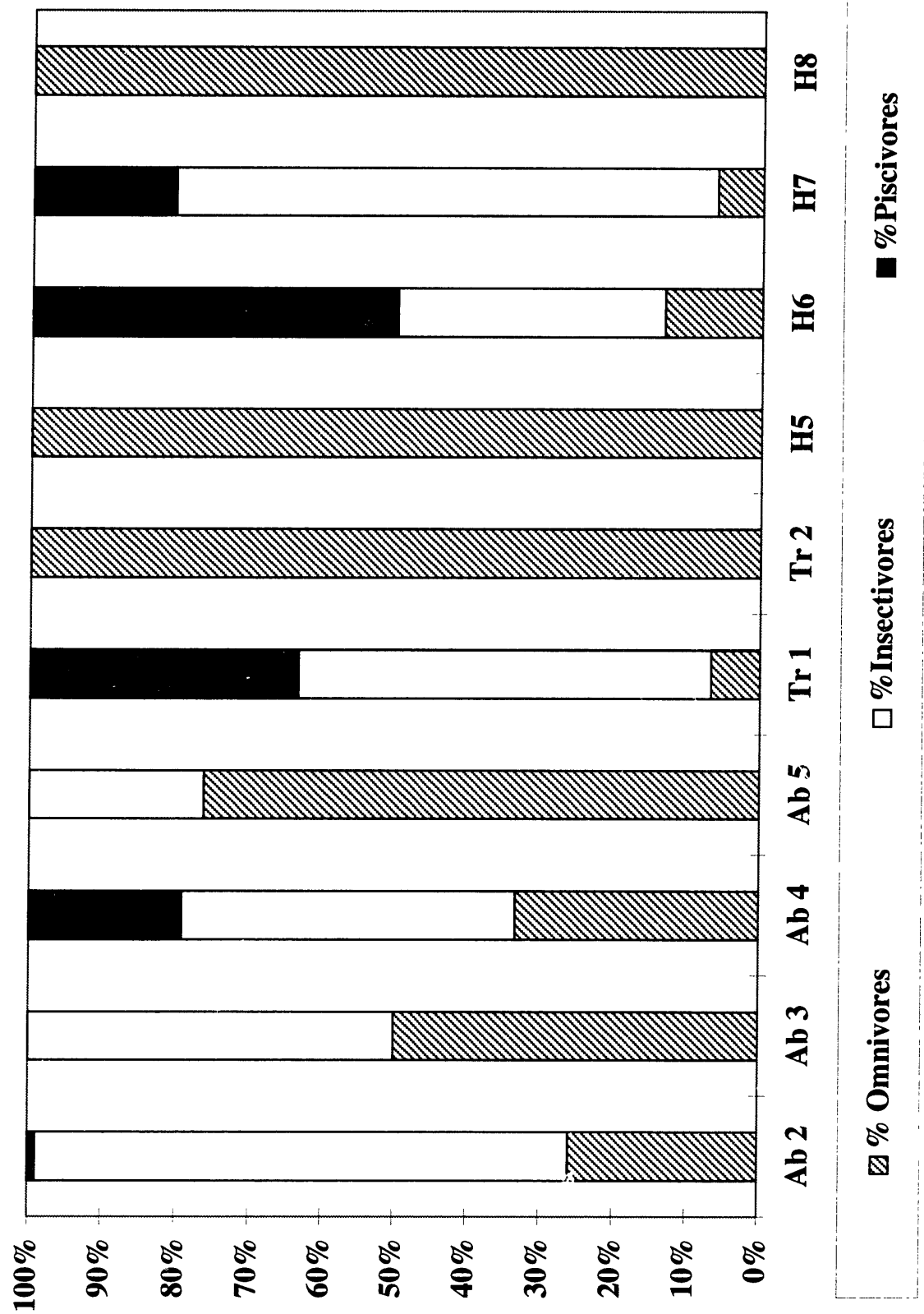
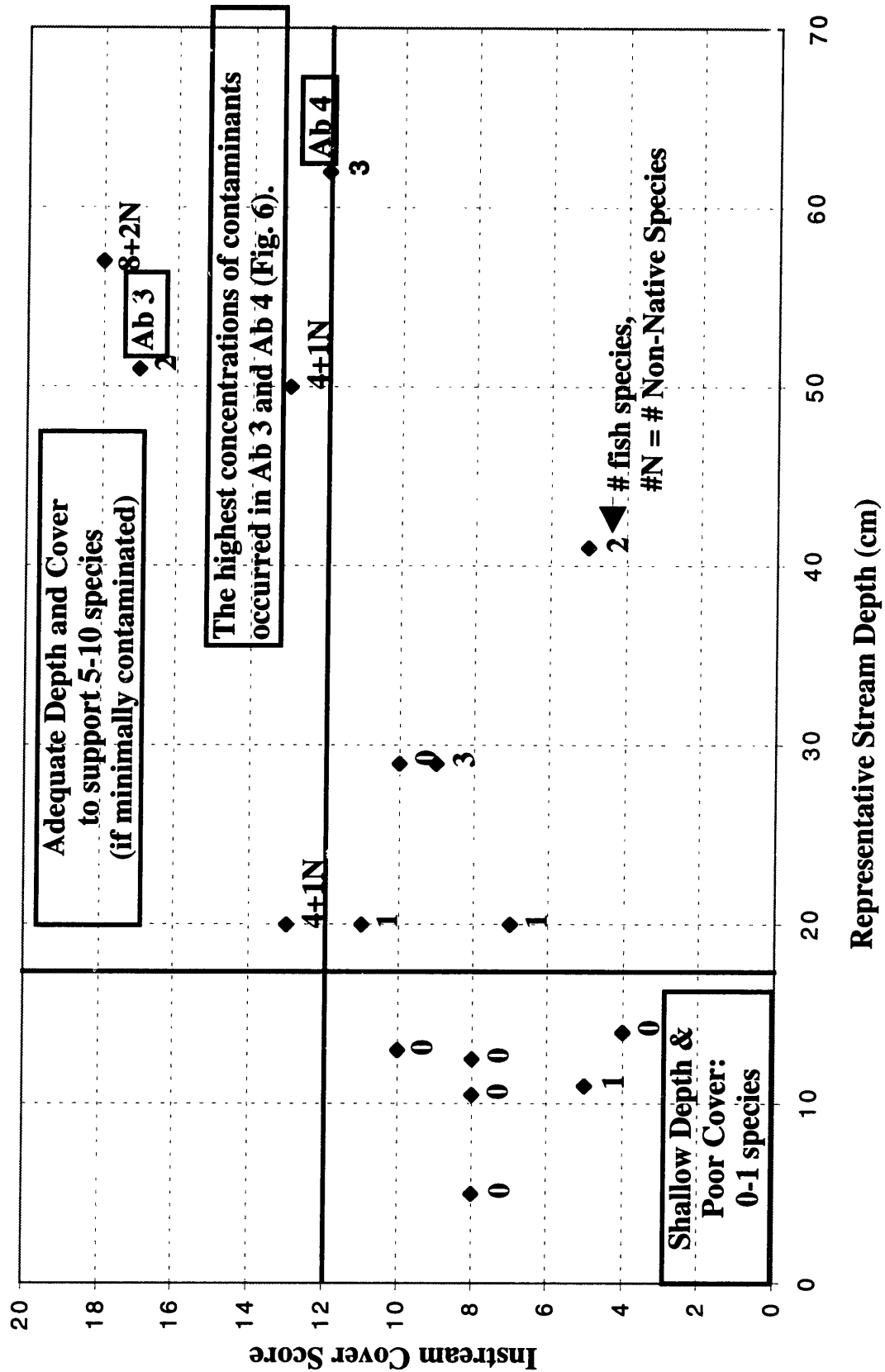


Figure 3.4. Relationship of the Number of Fish Species Caught at a Site (written below each point) to Instream Cover Score (y-axis) and Stream Depth (x-axis)



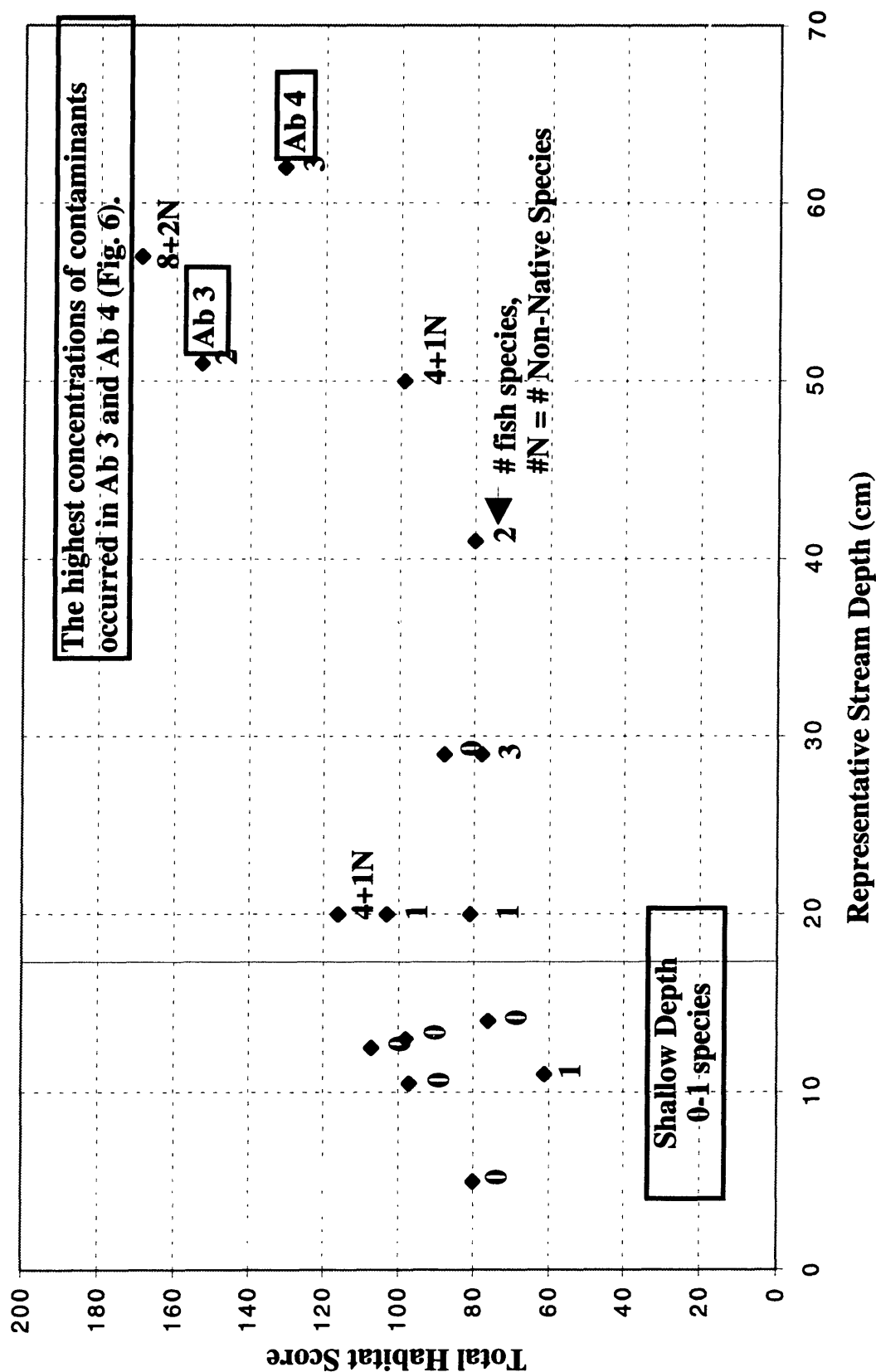
20 cm had instream cover scores of 10 or less. At five out of six of these sites, no fish were caught. At one out of six of these sites, one species was caught at an abundance of 10 fish per 1000 square meters (the lowest non-zero abundance of fish observed at any site).

The five sites with instream cover scores of 5-11 and stream depths of 20-45 cm had 0 to 3 fish species. No fish were caught at the site (Ab 1) located just south of the Industri-Plex 128 Superfund site in the center of a two-lane road. The narrow stream channel is bordered by steep, artificial banks and is intermittently covered by road crossings. One species of fish was caught at site H8 (abundance = 20 per 1000 square meters; instream cover score of 7) and site H5 (abundance = 142 per 1000 square meters; instream cover score of 11). Two species of fish were caught at site Ab 5. Three species of fish were caught at Tr 1, but 73% of the fish caught at this site were redbfin pickerel.

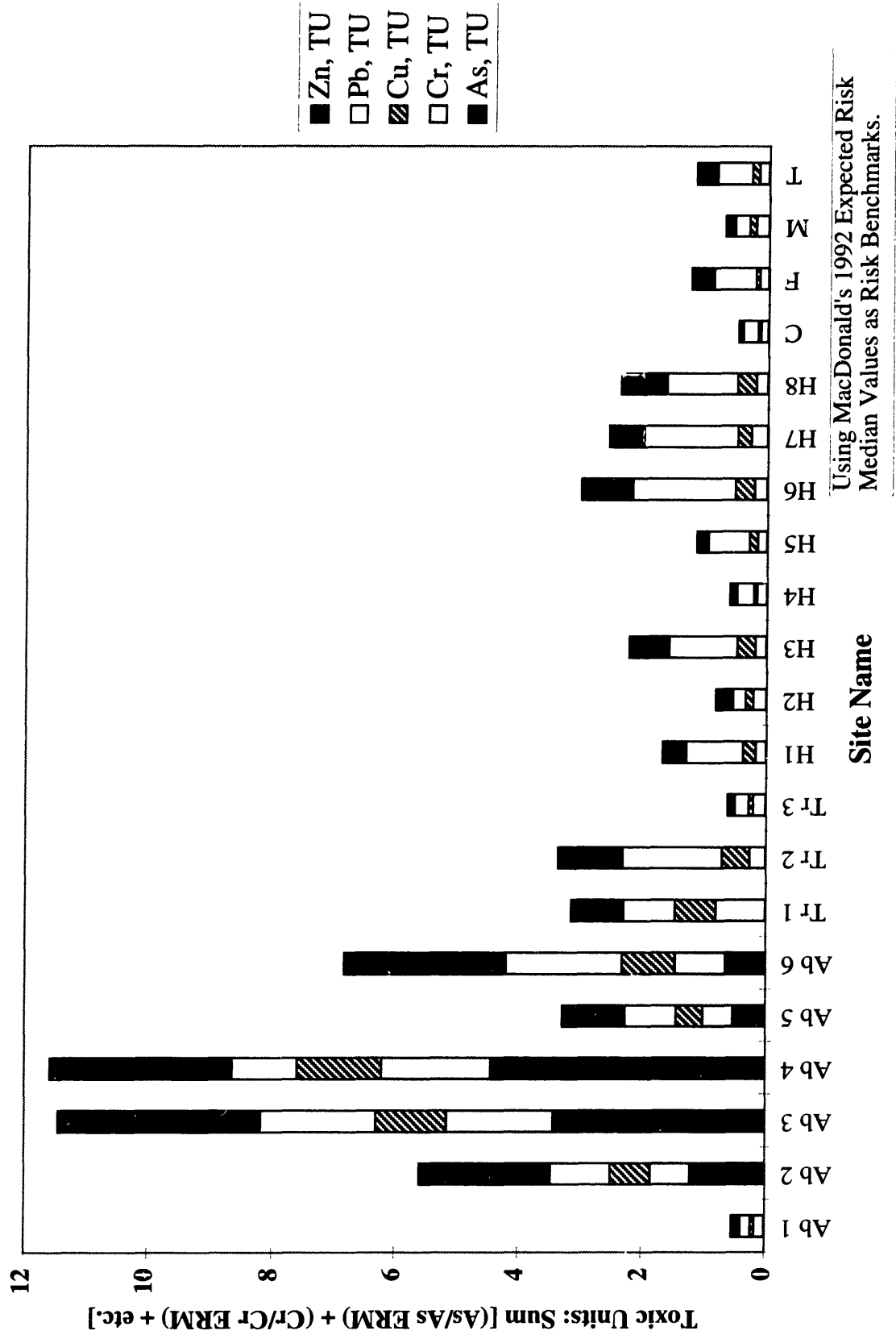
The five sites with instream cover scores of at least 12 and stream depths of at least 20 cm supported the greatest fish diversity. Two of these sites (H6, Ab 2) supported 4 native and 1 non-native species, and one site (H7) supported 8 native and 2 non-native species. The other two sites (Ab 3, Ab 4) supported only 2-3 species. These sites were highly contaminated (Fig. 3.6). The relationship of fish diversity to total habitat score was weaker than the relationship of fish diversity to instream cover score (Fig. 3.5).

The sediments and epifaunal habitat of sites located on the Aberjona River (Ab 1-Ab 6) were clearly more contaminated by As, Cr, Cu, Pb and Zn than other sites (Tr 1-3, H1-8) (Fig. 3.6). The method for fish sampling did not work at site Ab 6, so site Ab 6 was excluded from further discussion. Toxic unit scores (the sum of the ratios of each element divided by the ERM for that element) were highest for sites Ab 3 and Ab 4. These scores reflect that the concentrations of As and Zn in particular were much higher at these sites than at other sites. Pb concentrations were elevated (above ERM, reflected by a ratio >1) at many locations in the watershed. Zn concentrations in epifaunal habitat samples were higher than the ERM for Zn in sediment at some non-Aberjona River sites. Toxic unit

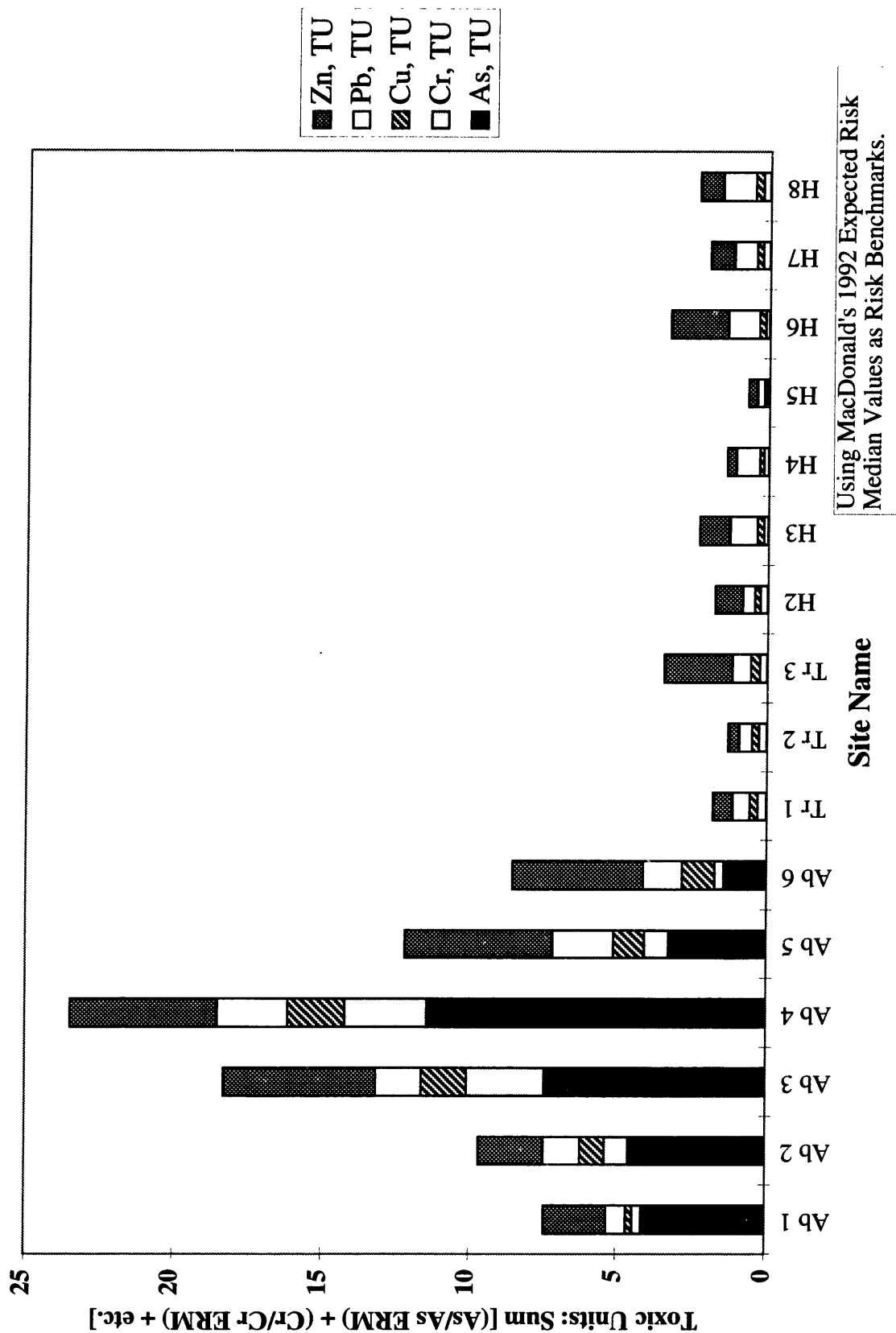
Figure 3.5. Relationship of the Number of Fish Species Caught at a Site (written below each point) to Total Habitat Score (y-axis) and Stream Depth (x-axis)



**Figure 3.6.a. As, Cr, Cu, Pb, Zn Sediment Contamination
Toxic Units vs. Sites**



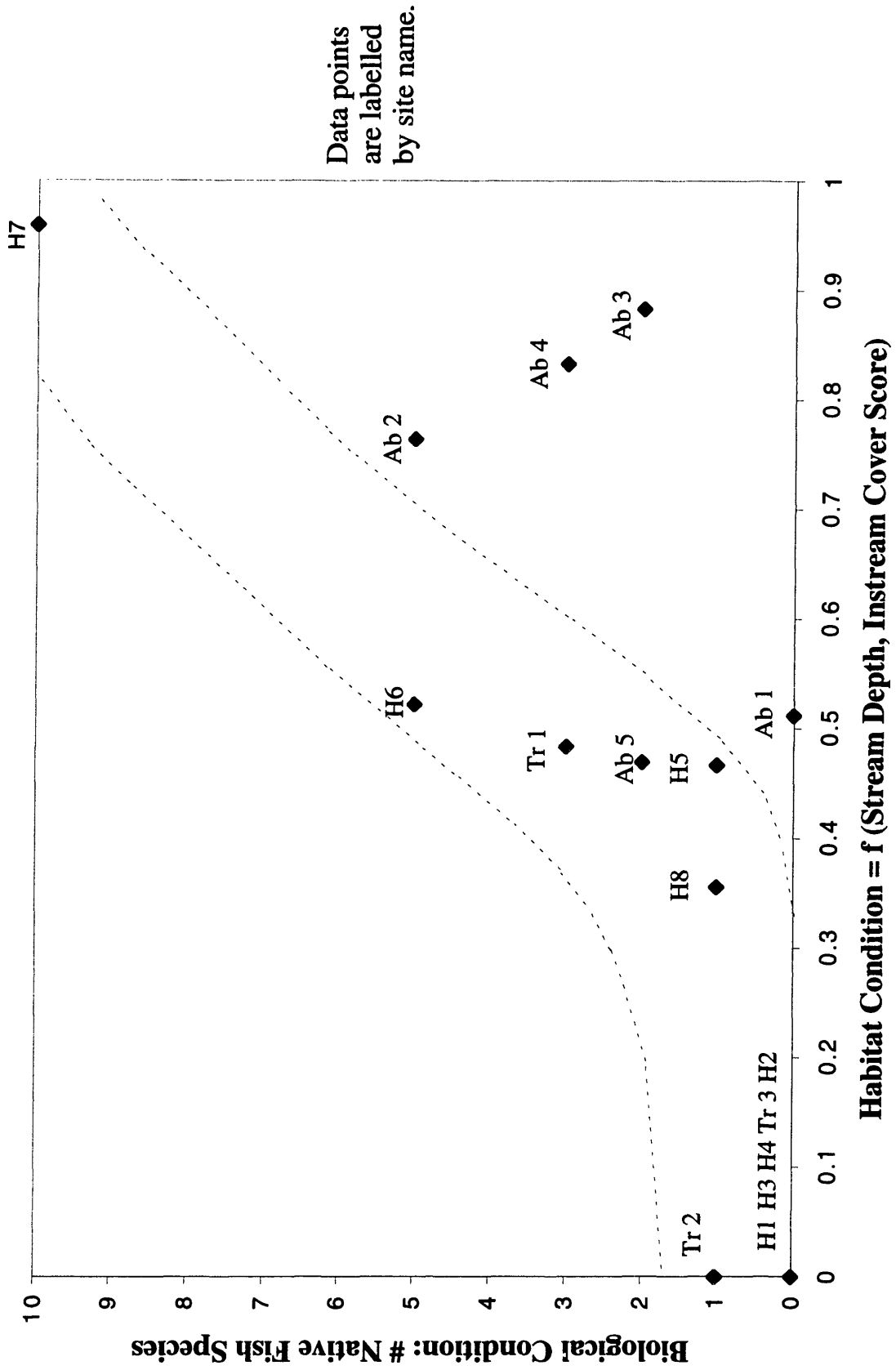
**Figure 3.6.b. As, Cr, Cu, Pb, Zn Epifaunal Habitat Contamination
Toxic Units vs. Sites**



scores were higher in epifaunal habitat samples than in sediment samples. The differences between toxic units scores for Ab 3 and Ab 4 and other sites were more pronounced in the epifaunal habitat samples.

The number of native fish species caught at each site was plotted versus each site's habitat condition score in Figure 3.7. The design of the figure was based upon the graphical conceptual model of Barbour *et al.* (1997) (Fig. 3.2). Using number of native fish species as the measure of biological condition and the habitat condition index developed specifically for this study, the expected regions of the graph were found. The graphical conceptual model of Barbour *et al.* (1997) does not specify the exact placement of the lines dividing the three regions. As predicted by Barbour *et al.* (1997), there were no sites with good biological condition (large number of fish species) and poor habitat condition. Sites H1, H3, H4, Tr 3, H2, and Tr 2 had poor biological condition and poor habitat condition. In these cases, the habitat condition score of 0 was the result of the empirically derived stream depth threshold (20 cm) for supporting fish species. Sites H5, H6, H8 and Tr 1 had intermediate biological condition (1-5 fish species caught at each site) and intermediate habitat condition scores and low levels of contamination (Fig. 3.6b); this is consistent with the Barbour *et al.* (1997) model. Site H7 had the highest number of species caught and the highest habitat condition score, which is consistent with the model. Sites Ab 1, Ab 2, and Ab 5 had intermediate toxic units scores, and sites Ab 3 and Ab 4 had high toxic units scores. Thus, sites Ab 1, Ab 2 and Ab 5 were predicted to have somewhat poorer biological condition than would be predicted by their habitat condition score, and sites Ab 3 and Ab 4 were predicted to have greater biological degradation attributable to contamination than sites Ab 1, Ab 2 and Ab 5. This relationship appears to have held for sites Ab 1 and Ab 2, but for sites Ab 1, Ab 2 and Ab 5 it was difficult to distinguish the impacts of contamination from the impacts of physical habitat degradation. Sites Ab 3 and Ab 4 have clearly lower biological condition than would have been predicted by their habitat condition scores; this is consistent with the model.

Figure 3.7 Relationship of Biological Condition to Habitat Condition



Metal Concentrations in Fish (Fig. 3.8, 3.9)

Concentrations of As in white suckers captured for this study at sites Ab 2, Ab 4 and Ab 5 were high (3-13 ppm) relative to white suckers caught at sites H5 and H6 (<1 ppm) (Fig. 3.8a). The highest concentrations (8-13 ppm) of As were measured in 3 fish caught at site Ab 2, whereas the other 6 fish caught at this site have concentrations of As in the same range (3-5 ppm) as the fish caught at sites Ab 4 (4-6 ppm) and site Ab 5 (2-5 ppm).

The concentrations of As in 6 of the white suckers captured for the U.S. EPA (data supplied by Mary Garren, U.S. EPA, Region 1) at nearby locations had similar concentrations (2-5 ppm) while 4 had significantly lower concentrations (<1 ppm). (The EPA sites are labeled in the figure according to their relationship to sites used in this study: site Ab2.b.EPA is located downstream of site Ab 2; site Ab.4.b.EPA is located downstream of site Ab 4; site Ab 5.b.EPA is located downstream of site Ab 5; site Ab 5.c.EPA is located downstream of site Ab 5.b.EPA; site Ab 6.EPA is located downstream of site Ab 6.) Concentrations of As in white suckers caught for the U.S. EPA at sites further downstream (Ab 5.c.EPA, Ab 6.EPA) and at a Horn Pond subwatershed site were consistently lower (< 1 ppm).

These data indicate that As was elevated in white suckers of the Aberjona River from a location just downstream of Industri-Plex 128 (site Ab 2) to a location some distance downstream of the Wells G & H Superfund site (sites Ab 5 and Ab 5.b.EPA), and dropped to background watershed levels further downstream (sites Ab 5.c.EPA, Ab 6.EPA).

The highest concentrations of Cr (8 ppm) occurred in white suckers from sites Ab 4 and Ab 5 (Fig. 3.8b). The concentrations of Cr in one white sucker from Ab 2 and one from H6 were also high (> 4 ppm). At most other sites, Cr concentrations were generally below 2 ppm.

Figure 3.8.a. [Arsenic] in White Suckers

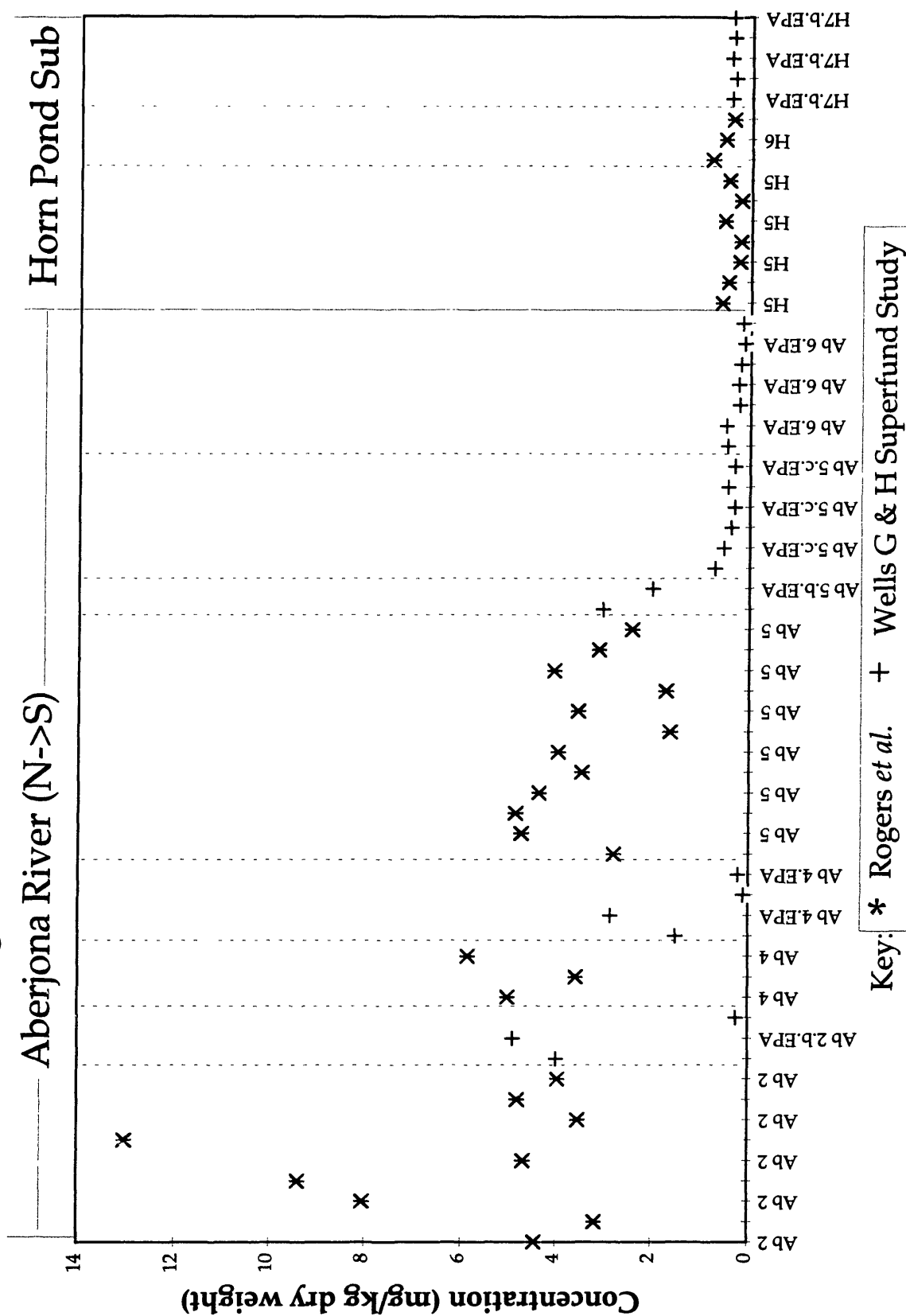


Figure 3.8.b. [Chromium] in White Suckers

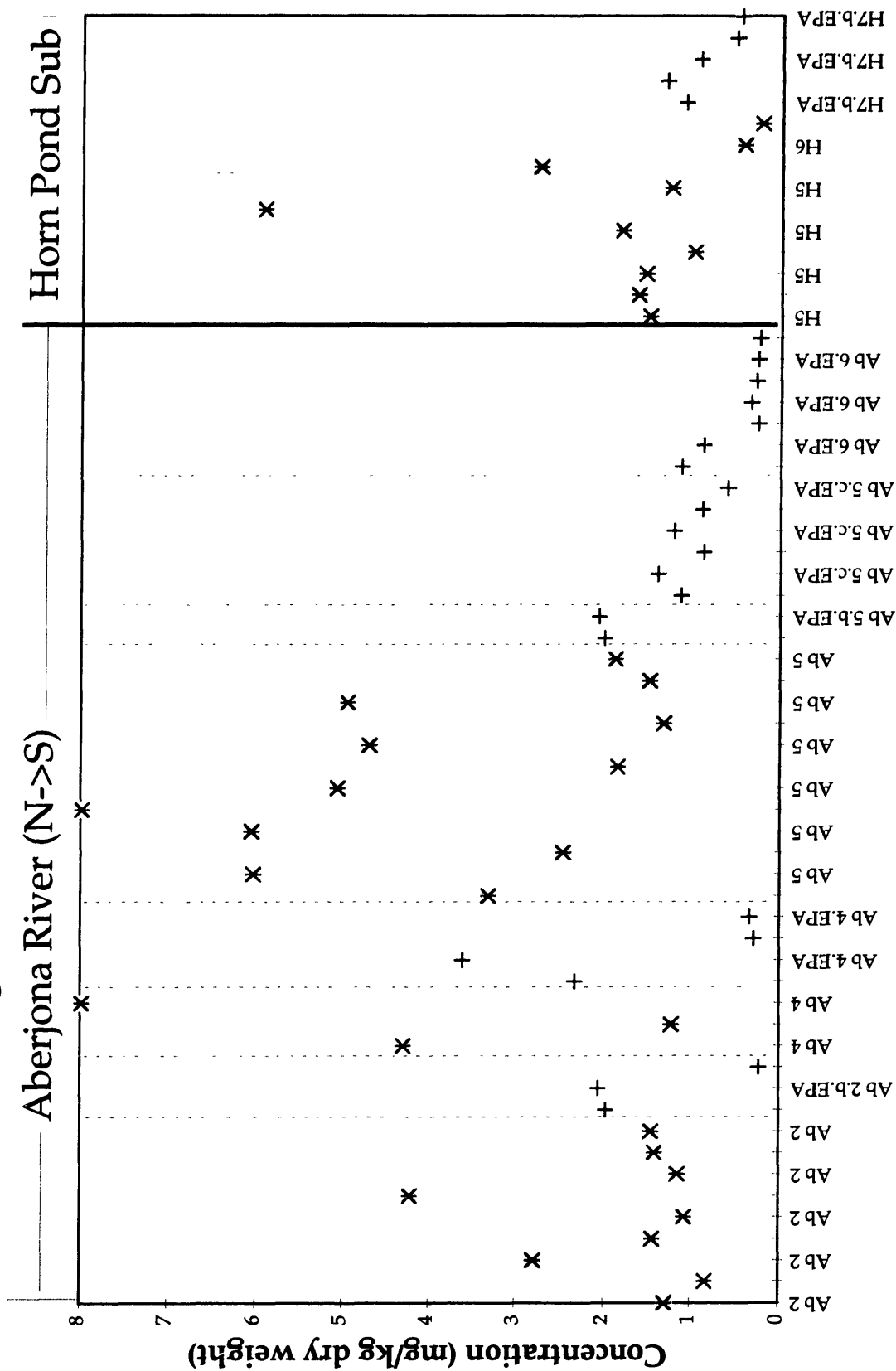
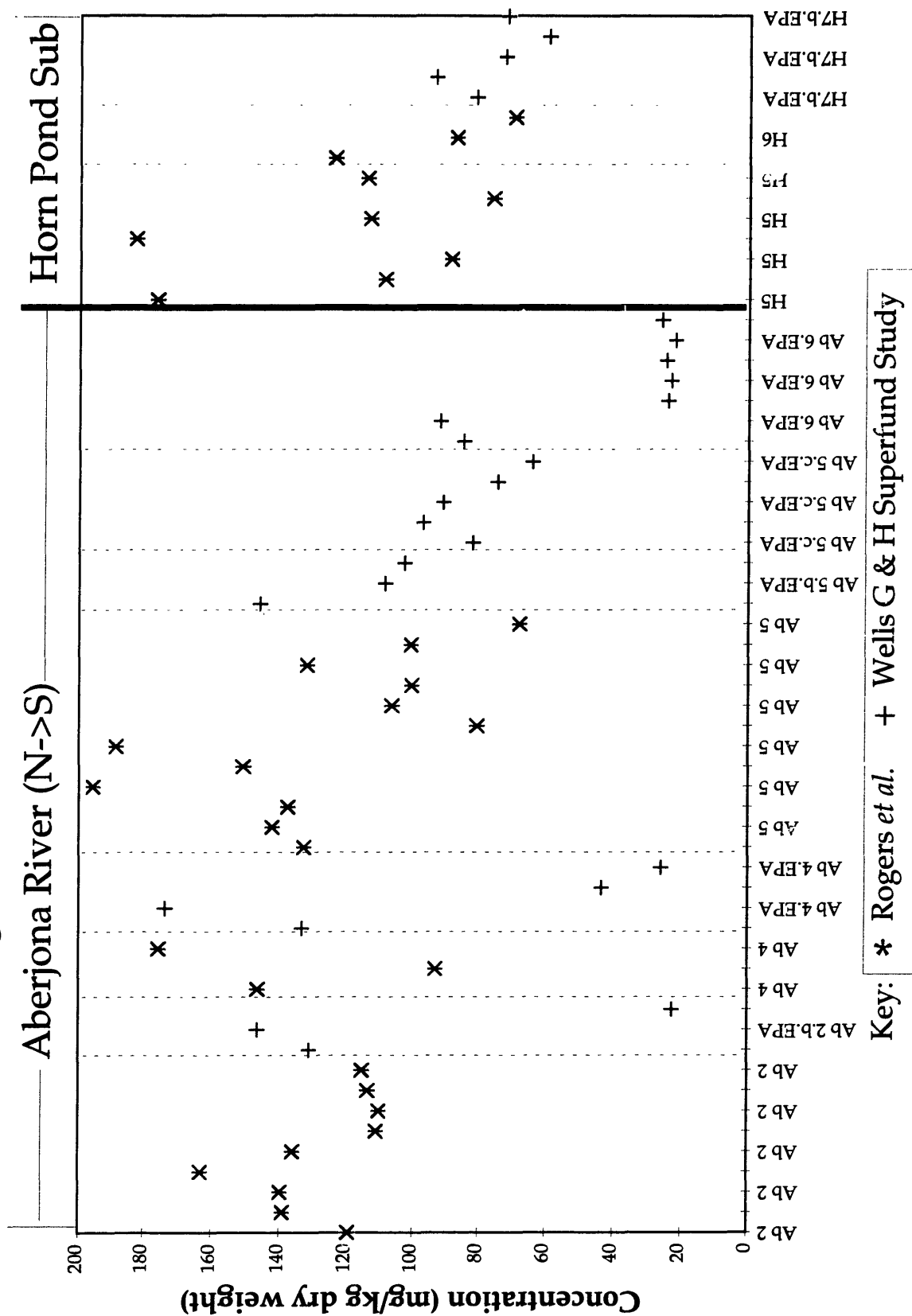


Figure 3.8.c. [Zinc] in White Suckers



A wide range of concentrations of Zn (20-200 ppm) in white suckers was measured (Fig. 3.8c). The lowest concentration that we measured was 65 ppm; the concentrations of Zn ranged from 20-45 ppm in 8 white suckers captured for the U.S. EPA. The full range of Zn concentrations measured in our study occurred at site Ab 5 (65-200 ppm), indicating the high variability of concentrations within a site. Concentrations did not appear to follow a geographic pattern within the watershed despite the higher concentrations of Zn in sediment and epifaunal habitat of the Aberjona River.

White suckers from sites Ab 2, Ab 4 and Ab 5 had higher average concentrations (and higher standard deviations) of As than white suckers from sites H5 and H6 (Fig. 3.9a). In addition, at sites Ab 2, Ab 4 and Ab 5, concentrations of As appeared to be greater in white suckers than in pumpkinseed or redbfin pickerel. Average concentrations of Cr in white suckers from sites Ab 2, Ab 4, Ab 5 and H5 were higher than in white suckers from H6; concentrations of Cr in white suckers were generally higher than concentrations of Cr in other species of fish (Fig. 3.9b). Standard deviation values were large, reflecting the high variability in concentrations of Cr in white sucker from one site. Average concentrations of Zn (all species) did not appear to follow a geographical pattern (Fig. 3.9c). Concentrations in white sucker and redbfin pickerel tended to be slightly larger than concentrations of Zn in pumpkinseed, but this difference was small relative to the standard deviation values for each species.

Concentrations of As, Cr and Zn were measured in both white suckers (Fig. 3.8) and in sediments/epifaunal habitat (Fig. 3.6) at five locations (Ab 2, Ab 4, Ab 5, H5, H6). Low concentrations of As and Cr were measured in sediment and epifaunal habitat samples from sites H5 and H6; higher concentrations of As and Cr were measured in both sample types at sites Ab 2, Ab 4 and Ab 5. Concentrations of As followed the pattern of high concentrations in white suckers from sites Ab 2, Ab 4 and Ab 5 and low concentrations in white suckers from sites H5 and H6. Beyond this simple relationship, concentrations of As in sediment and epifaunal habitat were not predictive of concentrations in fish. The

Figure 3.9.a. [As] in fish

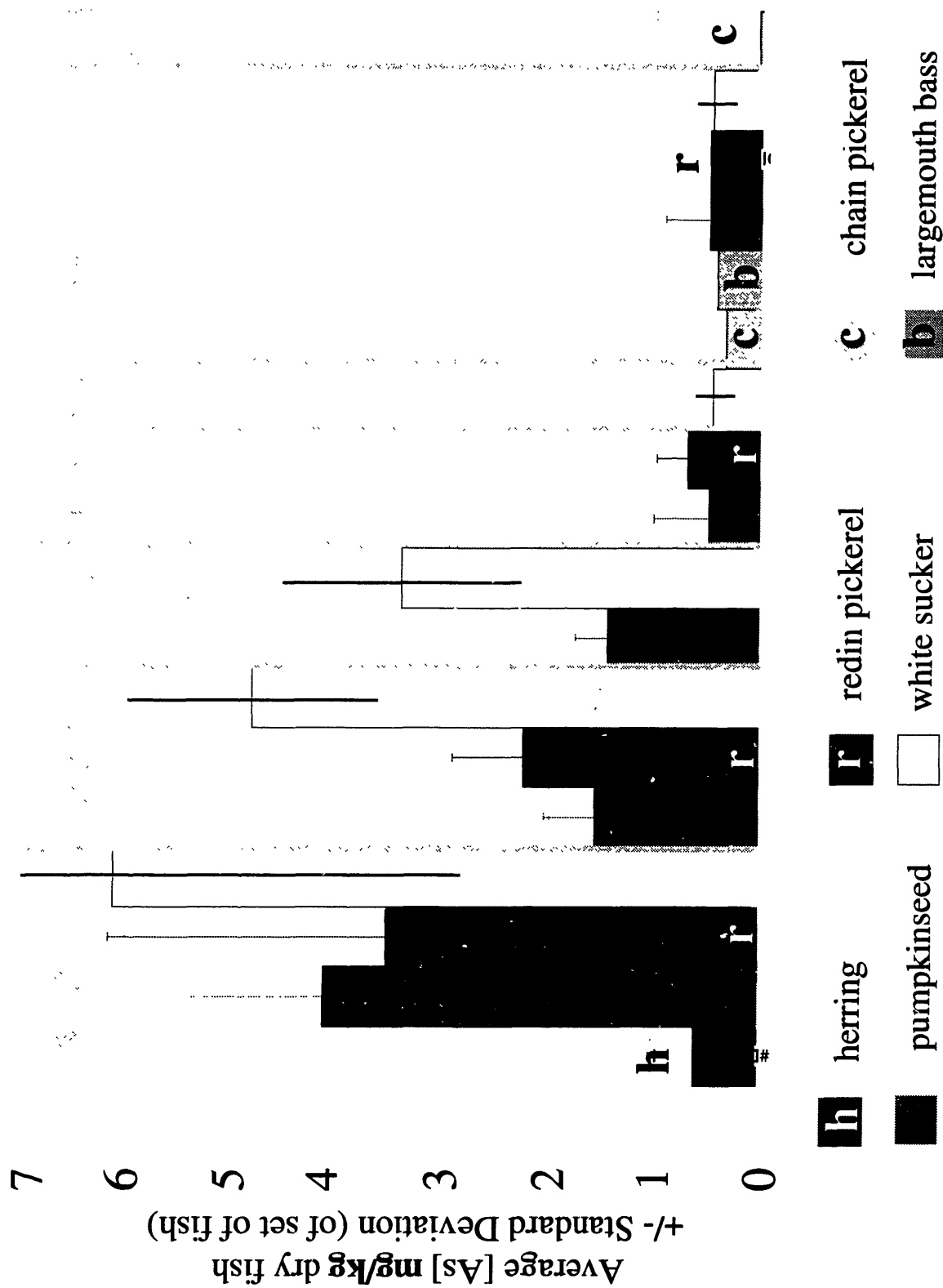


Figure 3.9.b. [Cr] in fish

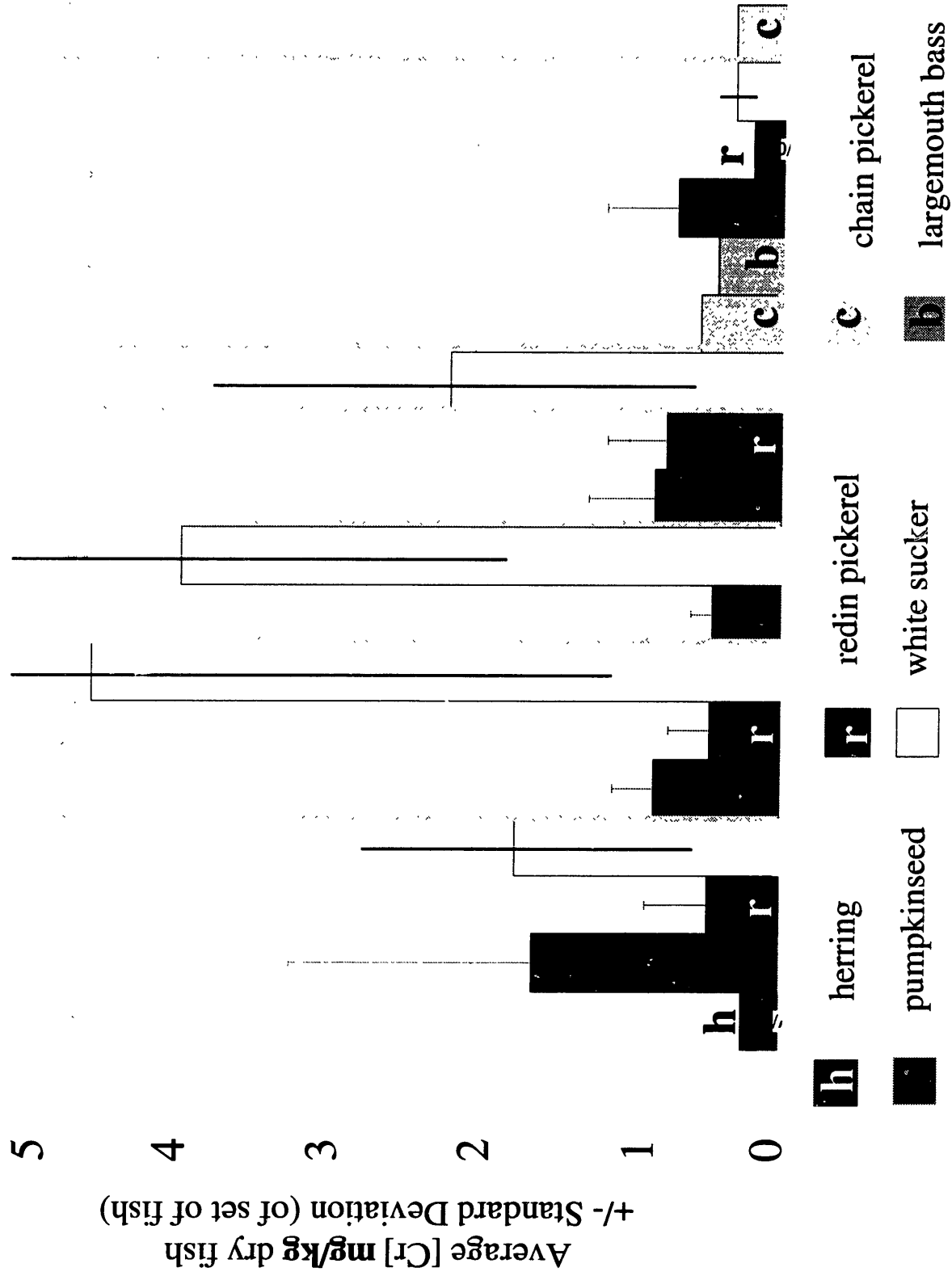
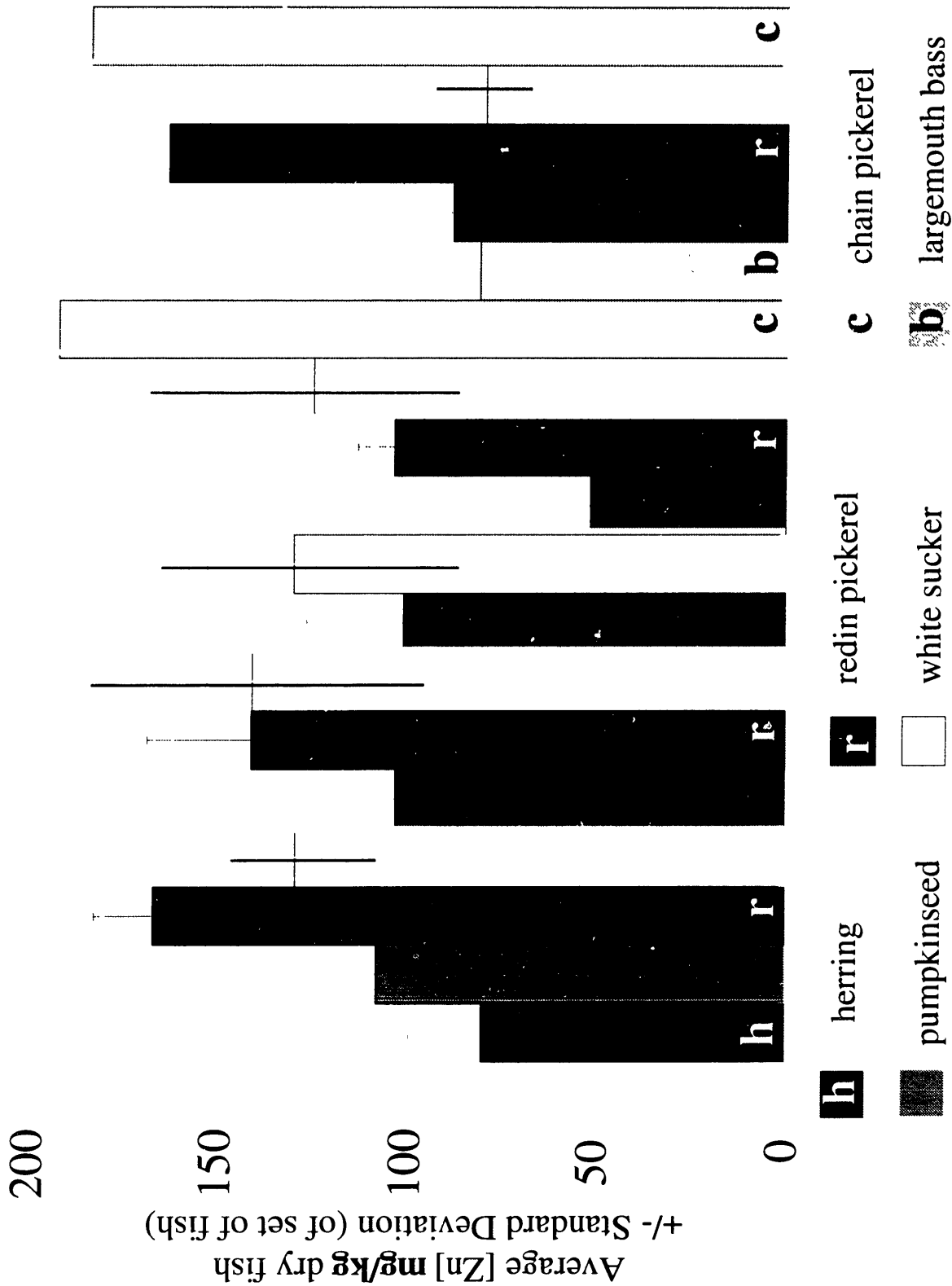


Figure 3.9.c. [Zn] in fish



concentrations of Cr in white suckers from sites Ab 2, Ab 4 and Ab 5 were generally higher than the concentrations in white suckers from sites H5 and H6. The difference between these two sets of sites was less pronounced than it was for As; the concentrations of Cr in one white sucker from H5 and one from H6 were close to the concentrations measured in fish from the Aberjona River. Concentrations of Zn in white suckers did not appear to be correlated with concentrations of Zn in epifaunal habitat or sediment samples.

Discussion

As predicted, we found that: (a) in the absence of contamination, biological condition varied from good to poor as habitat condition varied from good to poor, and (b) the presence of high concentrations of As, Cr, Cu, Pb and Zn in sediments and epifaunal habitat samples (high total toxic units scores) was associated with biological degradation beyond that predicted by habitat condition alone. Predictions were tested using the graphical framework developed by Barbour *et al.* (1997), and measures of biological condition, habitat condition, and chemical contamination selected and/or developed for this study. The number of fish species caught at a site was an unusually informative measure of biological condition, and was used to evaluate the relationship of biological condition to habitat condition and chemical contamination.

The results supported our hypotheses that stream depth and instream cover score were important components of habitat condition, but only a weak relationship was found between the number of species caught and the total habitat score. Shallow (<20 cm) stream reaches generally did not support fish. Fish require sufficient water depth to swim without becoming stranded, and benefit from water depths adequate to submerge a variety of habitats for feeding, spawning and refugia. Deeper (>20 cm) stream reaches, with adequate instream cover (scores > 12), supported 5-10 species in minimally contaminated areas. The relationship of instream cover to fish diversity (as reflected in this study by

number of species caught at a site) has been discussed by numerous authors and summarized by Tetra Tech (1995):

Instream cover includes the relative quantity and variety of natural structures in the stream, such as fallen trees, logs, and branches, large rocks, and undercut banks, that are available as refugia, feeding, or sites for laying eggs. A wide variety and/or abundance of submerged structures in the stream provide the fish with a large number of niches, thus increasing the diversity. As variety and abundance of cover decreases, habitat structure becomes monotonous, fish diversity decreases, and the potential for recovery following disturbance decreases.

The availability of instream cover in the Aberjona watershed has been decreased by urbanization. Stream banks have been developed in many areas for residential, commercial and industrial uses. The removal of stream bank vegetation, especially trees, has diminished the quantity of fallen trees, logs and branches in the streams. The importance of instream cover to fish diversity was suggested by spatial gradients in instream cover and the number of fish species in this study.

Other researchers have observed changes in fish assemblages due to the impacts of urbanization. Steedman (1988) observed that “comparative studies have shown strong degradative influences of agricultural and urban land use on the diversity of fishes and other biota of streams” (citing Larimore and Smith 1963, Tramer and Rogers 1973, Ragan and Dieteman 1975, Klein 1979, Goldstein 1981, Karr *et al.* 1986, Scott *et al.* 1986). In his study of the fish fauna of 209 stream locations in 10 watersheds near Toronto, Ontario, Steedman (1988) found a relationship between the Index of Biotic Integrity (IBI, adapted for regional use by Steedman as part of the study) and urbanization. (Steedman’s regression equation: $IBI = 29 - 19*URB + 14*RIP$; $r^2=0.7$, $n=18$, URB = proportion of basin in urban land use, and RIP =proportion of order 1-3 channels with intact riparian forest.) Weaver and Garman (1994), attributed changes in fish assemblages (lowered species diversity, lowered abundance of all species and guilds, and changes in feeding ecology) between 1958 and 1990 to road and bridge construction, commercial and residential development and riparian losses that occurred during that period.

Urban development can alter flow regimes in ways that are harmful to fish assemblages (Karr *et al.* 1986). Rain falling on a vegetated surface is absorbed into the ground and slowly released to streams. Rainfall in urban areas runs off paved surfaces and sharply increases stream flow during a storm, and does not get slowly released to maintain base flow during nonstorm conditions. Low channel flow status was observed at many locations in the Aberjona watershed, and it is likely that the urbanization of the Aberjona watershed has decreased base flow and increased peak flow during storms (chapter 2). In addition to this general effect of urbanization, stream flow has been severely reduced downstream of Horn Pond by groundwater wells pumping from an aquifer hydrologically connected to Horn Pond. The effect of pumping has been to reduce stream flow so drastically that site H8, which is located downstream of Horn Pond, had a measured stream depth of 20 cm in October 1995 and August 1996 and was completely dry in several sections in August 1997. Site H7, in contrast, which is immediately upstream of Horn Pond, has a water depth of greater than 50 cm and supported 8 native and 2 non-native species of fish. Site H8, which has been doubly impacted by reduced stream flow and physically degraded habitat (urbanization at this site has resulted in an instream cover score of 7), supported only a few white suckers in October 1995.

Despite good stream depths and instream cover scores, only 2-3 fish species were caught at the most severely contaminated sites (Ab 3, Ab 4). Contamination could be negatively impacting fish diversity in the Aberjona River. Effects of contaminated sediments that have been demonstrated in the laboratory include altered molecular and cellular functions, mortality, reproductive failure, reduced growth, poor condition and altered behavior (Environment Canada 1991). For example, Bryan and Langston (1992) reviewed the effects of As-contaminated sediments upon fish: studies of fish have indicated that reduced growth and inhibition of hemoglobin production can result from diets containing as little as 10 µg/g (As III), and toxic effects were observed in clams and fish following experimental exposure to As-contaminated sediments (other contaminants were

also present and could not be ruled out as the causative agents of toxicity). Munkittrick and Dixon (1988) observed reduced growth in females after sexual maturation, decreased egg size and fecundity, no significant increase in fecundity with age and an increased incidence of spawning failure in a white sucker population from a lake with elevated levels of Cu and Zn relative to a lake with lower levels of Cu and Zn; they suggested that these effects were more closely related to a decrease in available food than to metal accumulation in the fish. Dawson *et al.* (1988) found that zinc-contaminated sediments could cause teratogenicity, reduced growth and mortality in fathead minnow and frog embryos.

Contamination of stream sediments and epifaunal habitat by As and Cr was reflected in elevated concentrations of these metals in white suckers. Concentrations of Zn in fish were not related to sediment and epifaunal habitat contamination. Bendall-Young and Harvey (1989) found positive correlations between Zn concentrations in contaminated sediments and in fish livers; they noted that correlations had not been found between Zn concentrations in sediments and in 'whole' fish (they cited Johnson 1987), and suggested that liver concentrations were more reliable indicators of environmental concentrations.

It is usually difficult to separate chemical impacts from nonchemical impacts. Similar population and community responses are observed when fish are exposed to a variety of contaminants, deteriorating habitat, competition, poor weather and overexploitation; as a result, almost all of the data on contaminant effects on wild populations and communities are based on strong circumstantial evidence, and the current state of our knowledge of cause-effect relationships between contaminant loadings and multi-species fish yields is abysmally deficient in most areas (Environment Canada 1991 citing Colby 1984, Mix 1985, Black 1988, Mac 1988, and Ryder 1988).

It is unlikely that differences in habitat among Aberjona River sites could be responsible for the differences in the number of fish species caught at each site. As an example, site Ab 3 received optimal scores for 6 out of 10 habitat parameters, including instream cover, and suboptimal scores in the other 4 categories (chapter 2). Depths of 3,

51 and 81 cm were measured at three representative locations. The Horn Pond subwatershed site H7 had comparable habitat scores and stream depths. Site Ab 3 supported 20 fish (white suckers and pumpkinseeds) per 1000 square meters, while site H7 supported 120 fish of 10 different species.

Although the toxic units scores correspond to biological degradation in excess of that attributable to physical habitat degradation, contaminants other than those measured in this study could be responsible. Site Ab 3 is located immediately downstream of the Wells G and H Superfund site; other contaminants emanating from this site could be responsible. Site Ab 4 is located further downstream of the Superfund site. It is likely that the mechanism by which contaminants and physical habitat degradation exerted a negative impact upon fish diversity was complex, and may have involved factors not considered in this paper. Further study (e.g., laboratory or *in situ* toxicity testing) might help elucidate these relationships, but ecological complexity might prevent complete understanding of the impact of contaminants upon fish assemblages from ever being obtained.

Conclusions

Physical habitat quality, especially the availability of instream cover, was strongly related to fish diversity. Stream depth limited fish diversity in shallow stream reaches. This result is useful to managers because it facilitates the assessment of physical habitat degradation and contamination impacts upon fish. In addition, stream depth itself seems to have reflected the impacts of a disturbed hydrologic cycle that lowered base flow (and thus stream depth), and the disturbance of water withdrawals at Horn Pond that reduced streamflow downstream (e.g., at site H8). Contaminants appear to be reducing fish diversity downstream of the Wells G and H Superfund site, and elevated concentrations of As and Cr were found in white suckers at three sites downstream of the Industri-Plex 128 Superfund site.

This paper contributes to the understanding of the impacts of contamination (especially of sediments and epifaunal habitat) and physical habitat degradation (especially the urban/suburban degradation of small streams) upon fish assemblages. Perhaps more importantly, this paper demonstrates that bioassessment of fish assemblages can be interpreted with habitat assessment and contaminant evaluations to detect aquatic life impairment and to identify possible underlying chemical and physical causes. In addition, the method described in this paper could be used to regularly monitor improvements in chemical, physical and biological condition as remedial actions are taken.

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Chapter 4:

A Macroinvertebrate Index To Assess Cumulative Impacts to Urban Streams

C.E. Rogers, M.T. Barbour, H.F. Hemond, and D.H. Marks

Abstract. A multimetric approach was developed to characterize the biological condition of macroinvertebrate assemblages of an urban watershed (the Aberjona River watershed), and the cumulative impacts of physical habitat degradation and metal contamination of sediments upon macroinvertebrate assemblages were evaluated. Using the protocols of the Massachusetts Department of Environmental Protection (MA DEP), macroinvertebrates were sampled and physical habitat was assessed at 16 sites in the Aberjona watershed and 4 ecoregional reference sites (minimally-impaired locations in the same ecoregion as the Aberjona watershed). The MA DEP's multihabitat assessment protocols for macroinvertebrate sampling involve collection from submerged vegetation, vegetation-covered rocks, overhanging bank vegetation, and undercut bank roots. These "epifaunal" habitats, and fine-grained sediments (which are traditionally collected for risk assessments), were collected and analyzed for As, Cr, Cu, Pb and Zn. The highest concentrations of As, Cr, Cu, Pb and Zn were measured in epifaunal habitat samples, and the ranking of sites according to contamination level depended upon which sample type (sediments or epifaunal habitat) was used. Twelve benthic metrics were used to characterize biological condition and to relate biological impairment to chemical and physical degradation (using linear regression and *t-tests* comparing site categories). Sites were grouped according to level and type of chemical and physical degradation. As predicted, benthic metric values for sites with chemical and physical degradation were poorer or indistinguishably different from benthic metric values for reference sites. Severe physical degradation, moderate chemical and physical degradation, and severe chemical degradation were associated with similar levels of biological impairment. Individual benthic metrics were not diagnostic of impairment type. An aggregate macroinvertebrate index was developed using sensitivity to impairment, numerical discriminatory ability, non-redundancy, and inclusion of metrics from four categories (taxa richness, composition, tolerance and feeding measures) as criteria for selection. The aggregate macroinvertebrate index was more sensitive to chemical and physical degradation than *any* individual metric, thus illustrating the strength of a multimetric index approach for detecting the cumulative impacts of physical habitat degradation and chemical contamination.

Introduction

The objective of the Clean Water Act is to "restore and maintain the chemical, physical and biological integrity of the Nation's waters" [FWPCA §101, §1251(a)]. This objective poses formidable challenges in urban watersheds. One scientific challenge is the extension of existing methods - that have been developed to characterize the biological condition of freshwater streams, and to evaluate how chemical and physical stressors have

contributed to their biological impairment (e.g., Barbour *et al.* 1996a, 1996b, 1997, Gibson *et al.* 1996) - to the assessment of urban streams.

Benthic metrics are characteristics of benthic macroinvertebrate assemblages relating to species composition, diversity and functional organization that frequently change (usually in a predictable direction) in response to chemical and/or physical stressors (Barbour *et al.* 1997, p. 7-14 cite as examples: DeShon 1995, Shackleford 1988, Plafkin *et al.* 1989, Barbour *et al.* 1992, 1995, 1996a, Hayslip 1993, Kerans and Karr 1994, Fore *et al.* 1996). The use of benthic metrics to detect aquatic life impairment depends upon determining optimal benthic metric values; this is commonly accomplished by selecting several reference sites (sites that are not chemically or physically stressed) and calculating the distribution of benthic metric values for these sites. Four categories of benthic metrics encompassing several aspects of the structure and function of macroinvertebrate assemblages (Table 4.1) were described by Barbour *et al.* (1997): a) richness metrics, which represent the diversity within a sample, b) composition metrics, which reflect relative contributions of selected populations to total fauna, c) tolerance/intolerance metrics, which represent relative sensitivity to stressors, and d) feeding/habit metrics, which provide information on the proportion of organisms with particular feeding/habit strategies.

Table 4.1. Importance of Metric Categories

<u>Category</u>	<u>Explanation</u>
Richness	High diversity suggests that niche space, habitat, and food sources are adequate to support survival and propagation of many species. Subsets of total taxa richness (such as number of Ephemeroptera, Plecoptera and Trichoptera [EPT] taxa) are also used to accentuate key indicator groupings of organisms.
Composition	The premise underlying composition metrics (such as % EPT and % Orthocladiinae to chironomids) is that a healthy and stable assemblage will be relatively consistent in its proportional representation, though individual abundances may vary in magnitude.
Tolerance	The premise underlying tolerance metrics is that some organisms are more tolerant of a variety of stressors than other organisms. The percent Hydropsychidae to total Trichoptera is an estimate of evenness within an insect order that is generally considered to be sensitive to pollution. Dominance by tolerant Hydropsychidae is indicative of a stressed system.

Feeding/Habit Feeding metrics, such as % Filterers, number of scraper and piercer taxa are surrogates of complex processes such as trophic interaction, production, and food source availability (Karr *et al.* 1986, Cummins *et al.* 1989, Plafkin *et al.* 1989). Imbalances in functional feeding groups reflect stressed conditions. Specialized feeders, such as scrapers, piercers, and shredders, are the more sensitive organisms and are thought to be well represented in healthy streams. Generalists, such as collectors and filterers, have a broader range of acceptable food materials than specialists (Cummins and Klug 1979), and thus are more tolerant to pollution that might alter the availability of certain food. However, filter feeders are also thought to be sensitive in low gradient streams (Wallace *et al.* 1977).
Habit metrics, such as % clingers, are surrogate measures of behavior and modes of existence (Merritt and Cummins 1996).

A multimetric approach is recommended to characterize the biological condition of freshwater streams to provide detection capability over a broad range of stressors (Barbour *et al.* 1996b, Karr *et al.* 1986, Karr 1991, Plafkin *et al.* 1989, Gibson *et al.* 1996, Ohio EPA 1987). Ideally, to be included in an aggregate macroinvertebrate index, benthic metrics should: (a) be chosen from among the four categories (Table 4.1), (b) have the demonstrated ability to discriminate impaired from unimpaired conditions, and (c) not be redundant with other metrics (Barbour *et al.* 1996b, Gibson *et al.* 1996). A metric can discriminate impairment if the range of values for reference sites is distinguishably different (usually in a predictable direction) from the range of values for sites with known chemical or physical disturbances.

Characteristics of urban watersheds complicate the selection of reference sites and benthic metrics. Entire ecoregions can be influenced by human activity centered around a major city, making the selection of minimally-impaired reference sites challenging. Benthic metric values at such sites may not be truly optimal, although they represent the best biological condition in the ecoregion. In these cases, the number of intolerant taxa groupings (e.g., Ephemeroptera, Plecoptera, Trichoptera) may be low, making it difficult to distinguish lower values at impaired sites from the reference condition. Similarly, the percentage of tolerant organisms at reference sites may be variable or high, making it difficult to distinguish impairment (high values in this case) from the reference condition.

Macroinvertebrate assemblages in urban streams are typically subjected to a variety of chemical and physical stressors. Gibson *et al.* (1996) suggested that reference conditions different from natural systems may need to be established to adequately evaluate changes in biological condition that may be attributable to chemical impacts when major physical impacts (such as channelization of streams in urban areas) are present. We assert that the significance of this suggestion is that field studies of contaminant impacts (e.g., for research, management, and/or ecological risk assessment purposes) need to consider differences in habitat quality between sites.

This paper presents the multimetric approach that we developed to characterize the biological condition of macroinvertebrate assemblages of an urban watershed in Massachusetts (the Aberjona River watershed). Because the watershed is urban (land has been developed for residential, commercial and industrial purposes) and located within the Boston Basin ecoregion, the difficulties discussed above regarding the selection of reference sites and benthic metrics applied.

We also developed an approach to evaluate the cumulative impacts of chemical and physical stressors upon macroinvertebrate assemblages. The Aberjona River's chemical integrity has been compromised by two Superfund sites (U.S. Environmental Protection Agency National Priority Listed sites: the Industri-Plex 128 and Wells G & H sites). Thus, the attribution of biological changes to chemical and/or physical causes required an analysis of both types of stressors. Sites with varying degrees of chemical and/or physical stress were compared to sites with minimal stress (ecoregional reference sites), and to each other.

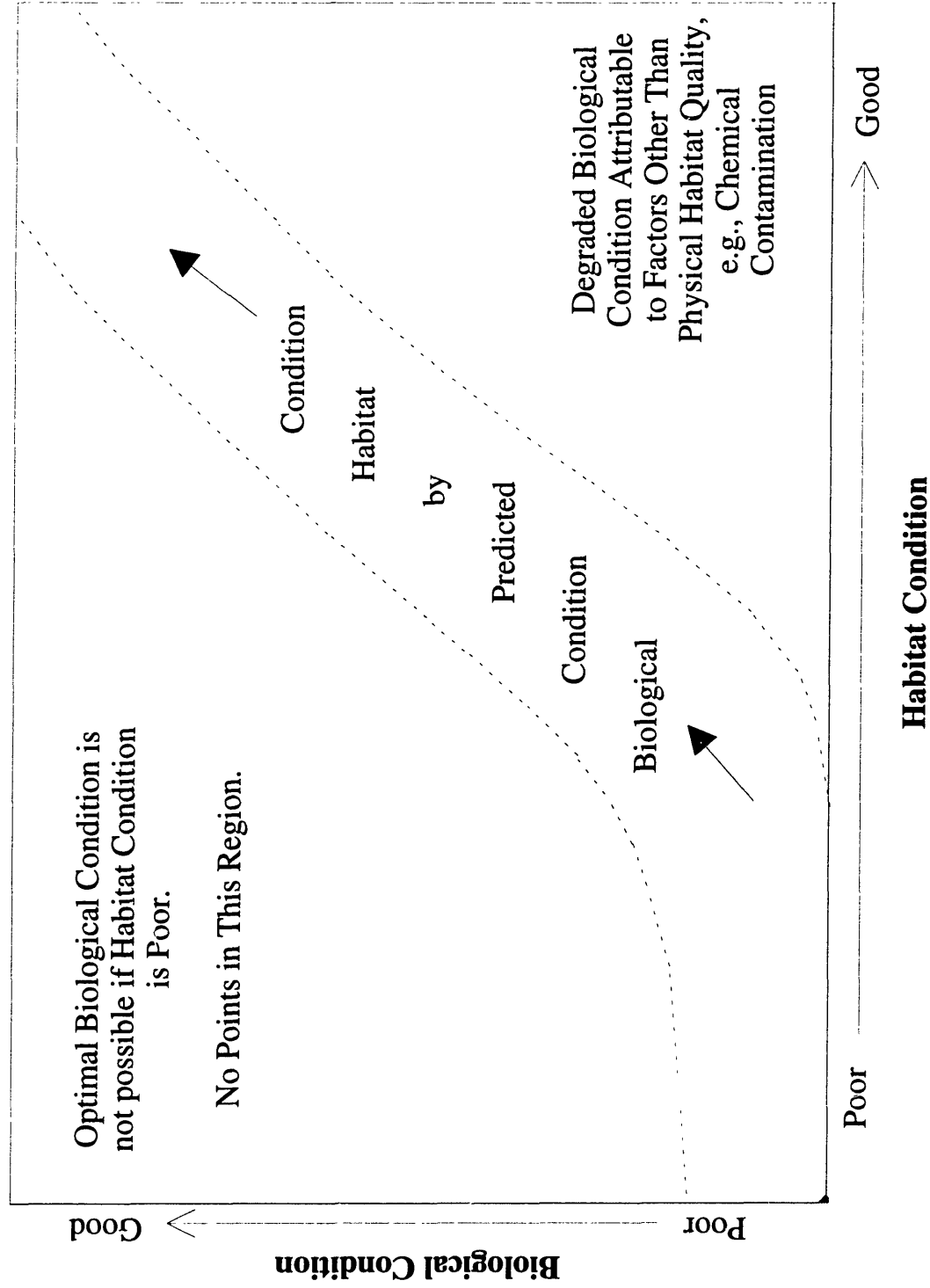
Long and MacDonald (1992) suggested that quantification of the relative likelihood of toxicity over specific ranges of chemical concentrations and assessment of potential ecological hazards of contaminated sediments is possible using threshold values developed by Long and Morgan (1990) and MacDonald (1992). The threshold called "Expected Risk Median" (ERM) is the median concentration associated with toxic effects (using laboratory spiked-sediment bioassays, equilibrium partitioning and field studies) (Long and Morgan

1990, MacDonald 1992, Long and MacDonald 1992). Concentrations of As, Cr, Cu, Pb and Zn in Aberjona River sediments exceed Long and Morgan's ERM values in many locations (chapter 2).

The model of Barbour and Stribling (1991) and Barbour *et al.* (1997) conceptually integrates chemical, physical and biological assessment (represented in Fig. 4.1). Barbour *et al.* (1997) hypothesized that in the absence of chemical contamination, biological condition varies from good to poor as habitat condition varies from good to poor. Biological condition cannot be good unless habitat condition is of a level to support a healthy biological community. Conversely, degraded biological condition in locations with good habitat can be attributed to something (e.g., chemical stressors) other than habitat quality (Barbour *et al.* 1996a, 1997). Barbour *et al.* (1997) also noted that it could be difficult to distinguish between habitat effects and other stressors if habitat condition were intermediate and biological condition were poor.

Thus, we hypothesized that: (a) in minimally contaminated aquatic environments of the Aberjona watershed, biological condition would vary from good to poor as habitat condition varied from good to poor, and (b) that in locations where concentrations of As, Cr, Cu, Pb and Zn in sediments exceeded ERM values, that biological condition would be degraded beyond expectations based upon habitat condition alone. Riffle habitats are scarce in the Aberjona watershed, and most macroinvertebrates inhabit submerged vegetation, vegetation-covered rocks, overhanging bank vegetation, and undercut bank roots. We hypothesized that the concentrations of As, Cr, Cu, Pb and Zn in these epifaunal habitats were more appropriate measures of the exposure of macroinvertebrates to contaminants than the concentrations of these elements in the fine-grained, organic rich sediments that are traditionally sampled for risk assessment purposes, and we developed a method for sampling these habitats.

**Figure 4.1 Conceptual Model of Barbour *et al.* (1997)
Relationship of Biological Condition to Habitat Condition**



Methods

Selection of Aberjona River Watershed Sites

Seventeen locations in the watershed were chosen for study (Fig. 1.a): 6 are located along the Aberjona River from its source to its end in the Mystic Lakes; 8 sites are located throughout the Horn Pond subwatershed that carries water from western part of the Aberjona watershed to the Aberjona River between Aberjona River sites Ab 5 and Ab 6; and 3 sites are located on the three largest remaining tributaries to the river. One site (H1) that is located in the Horn Pond subwatershed was not sampled for macroinvertebrates because it was dry in August 1996. Locations were chosen from a combination of U.S.G.S. maps (Reading, MA, 1987 and Boston, MA-North, 1985), street maps, and site visits. All sites are in walking distance (usually upstream) of road crossings (they were chosen to allow access for backpack electroshocking of fish). Four additional locations outside of the watershed were chosen as reference sites.

Selection of Reference Sites

The entire Aberjona watershed is influenced by heavy human-related development, and the watershed is part of the Boston Basin ecoregion (Griffith *et al.* 1994). The least-disturbed stream reach in the Aberjona watershed was selected as a watershed reference site. This site is located in the Horn Pond recreational area, and is referred to as site H7 (Fig. 1.a).

Additional reference sites were sought within the ecoregion (e.g., within least-disturbed areas of the Charles River Watershed and the Concord River Watershed). The criteria for the reference sites were: (1) relatively undeveloped headwaters, (2) no evidence of human alteration of the physical habitat, (3) wide (>18 m) vegetated riparian zones, and (4) no evidence of pollution (Hughes *et al.* 1990). Fifty candidate reference locations were selected from U.S.G.S. maps and from consultation with knowledgeable resource managers and scientists (personal communication with the Charles River Watershed

Association, Scott Socolosky of the Civil and Environmental Engineering Department of the Massachusetts Institute of Technology, the U.S. Army COE, the U.S.G.S., the Massachusetts Department of Environmental Protection, and the U.S. EPA New England Regional Lab in Lexington, MA). These locations were selected because their geological and hydrological properties (stream order, drainage area and gradient) were similar to the sites in the Aberjona Watershed (U.S. Geological Survey maps: Reading, MA, 1987 and Boston, MA-North, 1985) and because minimal disturbance was suggested by available information. Four sites (referred to as C, F, M and T) were determined to be of acceptable quality, and were chosen as “minimally-impaired” sites (Fig. 1.b).

Habitat Assessment

Physical habitat assessment was performed at 17 test sites and 4 reference sites (Fig. 1, see also chapter 2) in August, 1996, according to the protocols used by the Massachusetts Department of Environmental Protection (Tetra Tech 1995) modified for slow-flow, low-gradient streams. Ten habitat parameters (instream cover, epifaunal substrate, embeddedness, channel alteration, sediment deposition, variety of velocity-depth combinations, channel flow status, bank vegetative protection, bank stability, and riparian vegetative zone width) were scored from 0 to 20.

Sample Collection for Metal Analysis

After visual determination of the range of sediment grain size and organic content, areas of fine-grained, organic-rich sediments were collected at 17 test sites and 4 reference sites (Fig. 1) using a Russian corer. The torpedo-shaped, aluminum corer head was gently inserted into the top 2-3 cm of sediment and twisted 180° to capture the sample. The corer was pulled out of the sediment, twisted again to expose the sample, and sediment was scooped off the corer with a teflon spatula and stored in glass jars.

Epifaunal habitats (submerged vegetation, vegetation-covered rocks, overhanging bank vegetation, and undercut bank roots) were sampled and stored in glass jars. Watercress was sampled at sites Ab 1, H4, H5 and Tr 2. Submerged vegetation was sampled at sites Ab 3, H7 and T. Undercut bank roots were sampled at sites Ab 2, Ab 5, Ab 6, H3, H8 and Tr 3. Vegetation was scraped from rocks at sites H2, H3, H6, C and F. Silty overhanging bank vegetation was sampled at site Ab 4. A mixture of watercress and submerged vegetation was sampled at site Tr 1 and a mixture of emergent and overhanging bank vegetation was sampled at site M.

Jars containing sediment and habitat samples were immediately placed on ice and kept refrigerated (12 hours to 2 days) until they were dried at 85°C to a constant weight (approximately 1-2 days).

Metal Analysis

Five (5.0) grams of each dried sample were pulverized in a Spex Mixer cartridge with a silicon carbide ball for 5 minutes. One-half gram (0.5 g) of copolywax (TM) binder was added to the sample and mixed again for 1 minute. This mixture was poured into a 31mm diameter aluminum sample cup and pressed for 1 minute using 12 metric tons of force in an evacuable die. Elemental concentrations in pellets were analyzed by X-Ray Fluorescence (XRF) using a Philips PW1480 wavelength dispersive XRF and Uniquant data processing software (Uniquant 1992). NIST standard reference material #2709 (San Joaquin soil) was used to verify that the instrument was functioning properly. (Analyses of Precision and Accuracy described in chapter 2)

Toxic Unit Analysis

Concentrations of As, Cr, Cu, Pb and Zn were normalized by sediment quality benchmarks (Table 4.2, Expected Risk Low (ERL) and Expected Risk-Median (ERM) values, Long and Morgan 1990, MacDonald 1992). The resulting risk ratios (environmental concentration/ benchmark concentrations) are dimensionless, and are referred to as toxic units. Ratios in excess of 1 occur when concentrations in sediments are higher than benchmarks.

Table 4.2. ERL and ERM Values for As, Cr, Cu, Pb and Zn (MacDonald 1992)

	As	Cr	Cu	Pb	Zn
ERM (ppm)	70	370	270	223	410
ERL (ppm)	8.2	81	34	46.7	150

Macroinvertebrate Sampling

Macroinvertebrate sampling was performed at 16 locations (H1 was not included because it was dry at the time of sampling) in the Aberjona watershed and at 4 additional “reference” locations in August 1996 according to the protocols used by the Massachusetts Department of Environmental Protection (Tetra Tech 1995). The protocols are similar to Method 7.2 “Multihabitat Approach; D-Frame Dip Net” described by Barbour *et al.* (1997). Briefly, the multihabitat approach consists of sampling macroinvertebrate habitats, such as cobble, snags, vegetated banks, submerged macrophytes and sand, in proportion to their visually-estimated representation in the stream. The stream is visually assessed and rough percentages are assigned to the proportion of available instream habitat belonging to each category. Samples are collected by kicking the substrate or jabbing with a rectangular dip net (0.5 m width, 0.3 m height). A total of 10 jabs (or kicks) were taken from all major productive habitat types in the reach resulting in sampling of approximately 2.5 m² of habitat. In this study, duplicate samples were collected at 3 randomly chosen locations.

Macroinvertebrate Subsampling and Species Identification

Samples were sorted and subsampled in the laboratory according to the protocols used by the Massachusetts Department of Environmental Protection (Tetra Tech 1995). Whole samples (all material collected in the 10 jabs or kicks, including benthic macroinvertebrates, bits of vegetation, small pebbles and sand) were rinsed; large organic material was rinsed, visually inspected, and discarded. Samples were spread evenly across trays marked with grids, and subsampled randomly. Organisms were sorted under a dissecting microscope. Subsamples of 100 plus/minus 20 organisms preserved in 95% alcohol were identified to the lowest practical taxon, generally genus or species. A representative of every species was maintained in a reference collection.

Characterization of Biological Condition

Biological condition was characterized with benthic metrics selected from among four categories: taxa richness, composition metrics, tolerance/intolerance measures, and feeding measures (Table 4.1).

Barbour *et al.* (1997, p. 7-15) listed benthic metrics that were used as components of 11 indices (Invertebrate Community Index, Ohio EPA 1987, DeShon 1995; Rapid Bioassessment Protocols, Barbour *et al.* 1992 revised from Plafkin *et al.* 1989; Rapid Bioassessment Protocols for Arkansas, Shackleford 1988; Rapid Bioassessment Protocols, Hayslip 1993, Idaho, Oregon, Washington; Benthic Index of Biotic Integrity for the Tennessee Valley, Kerans and Karr 1994; Benthic Index of Biotic Integrity for southwestern Oregon, Fore *et al.* 1996; Stream Condition Index for Florida, Barbour *et al.* 1996b; Multimetric Benthic Index for Wyoming, Barbour *et al.* 1994; Benthic Comparison Index for Delaware Piedmont Streams, John Maxted, DNREC, personal communication). These indices were used for streams in Ohio, Arkansas, Idaho, Oregon, Washington, Tennessee, Florida, Wyoming, and the Mid-Atlantic Coastal Plains. Although these

indices were developed regionally, they are typically appropriate over wide geographic areas with minor modification (Barbour *et al.* 1995, 1997).

Five benthic metrics were used in more than half of the 11 indices listed by Barbour *et al.* (1997). % Filterers was the most widely used feeding measure (4/11 indices), thus these six metrics were selected as the general set of candidate metrics for the Boston Basin ecoregion (Table 4.3.a).

Table 4.3.a. General set of candidate benthic metrics

<u>Category</u>	<u>Metric (frequency of use)</u>	<u>Expected Response to Perturbation</u>	<u>Definition</u>
Richness	Total No. taxa (10/11 indices)	Decrease	Measures the overall variety of the macroinvertebrate assemblage
	No. EPT taxa (8/11 indices)	Decrease	Number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)
Composition	% EPT (6/11 indices used some variant of % EPT)	Decrease	Percent of the composite of mayfly, stonefly, and caddisfly larvae (taxa sensitive to pollution and other stressors)
Tolerance	% Dominant taxon (6/11 indices)	Increase	Measures the dominance of the single most abundant taxon.
	Hilsenhoff Biotic Index (7/11 indices)	Increase	Uses tolerance values to weight abundance in an estimate of overall pollution. Originally designed to evaluate organic pollution.
Feeding	% Filterers (4/11 indices)	Variable	Percent of the macrobenthos that filter FPOM from either the water column or the sediment

While only 2/11 indices used % EPT as a composition measure, four others used % Ephemeroptera, % Plecoptera, % Trichoptera and/or Ratio EPT/Chironomid Abundance. Thus, 6/11 indices included some sort of EPT composition measure, other composition

measures were used in at most 3 studies. % EPT was chosen over % Ephemeroptera, % Plecoptera and % Trichoptera because these three insect orders were present in low percentages (the median for reference sites < 20%) in the Boston Basin ecoregion; thus, a summed percentage was preferable. % EPT was chosen over the Ratio EPT/Chironomid because the ratio is less robust than the percentage (Chapman *et al.* 1992).

A supplemental set of benthic metrics (Clements 1991, Resh and Jackson 1993, Resh 1995, Fore et al. 1996, Barbour *et al.* 1997, Maxted et al. in prep,) was analyzed to calibrate the usefulness of particular metrics in an urban Massachusetts system (Table 4.3b). Benthic metrics were calculated for each macroinvertebrate sample (23 samples in all: a site from each of 20 sites, plus duplicate samples from 3 sites).

Table 4.3.b. Supplemental set of candidate benthic metrics

<u>Category</u>	<u>Metric</u>	<u>Expected Response to Perturbation</u>	<u>Definition</u>
Composition	% Orthocladiinae to chironomids	Increase	Percent of chironomids in the subfamily Orthocladiinae
Tolerance	% Hydropsychidae to Trichoptera	Increase	Relative abundance of pollution tolerant caddisflies (metric could also be regarded as a composition measure)
	No. of Intolerant Taxa	Decrease	Number of taxa with tolerance scores <= 3.
	% Tolerant Organisms	Increase	Percent of sample composed of organisms with tolerance scores >=7.
Feeding	No. Scraper & Piercer taxa	Decrease	Number of taxa feeding upon living plant material either by scraping periphyton or by piercing macrophytes.
Habit	% Clingers	Decrease	Percent of sample composed of organisms classified predominantly as clingers (Merritt & Cummins 1996).

Percent Orthocladiinae to chironomids was selected as a supplemental metric because it has been found to positively correlate with contamination by As, Cr, Cu, Pb and Zn in several descriptive studies of water pollution and to Cu and Zn in experimental studies (Clements 1991). Percent Hydropsychidae to Trichoptera (used in only 2 indices) was selected to account for the dominance of the relatively pollution tolerant caddisflies in the Boston Basin ecoregion. Hydropsychidae were found to be highly tolerant of heavy metals (Cr, Hg, Pb and Ni) (Clements 1991, citing Petersen & Petersen 1983).

While the Hilsenhoff Biotic Index (HBI) has been shown to be useful in many instances (Resh and Jackson 1993, Resh 1995), the assumptions used for the tolerance assignments may not be totally appropriate for various parts of the country. The tolerance assignments used for the fauna have not been comprehensively tested for the Boston Basin ecoregion. The tolerance assignments used for the HBI are based on a continuum or gradient that ranges from the most sensitive to the most tolerant and includes those that might be considered facultative in their tolerance. Two other metrics that focus only on the extremes of the tolerance range may be more appropriate, because they will be less subjective (Maxted et al. in prep). Investigators are more in consensus on those organisms that are most sensitive to perturbation, or most tolerant. Those organisms that are "in-between" are arguably more subjective. Therefore, consideration of metrics, such as number of intolerant taxa (a richness measure of the most sensitive organisms that decreases when perturbation increases) and percent tolerant organisms to the total fauna (a composition measure that increases when tolerant organisms become more dominant as sensitive organisms are lost), were adopted as candidate tolerance metrics.

Number of scraper and piercer taxa was included because taxa that feed upon living plant material are especially important and sensitive in streams in which plant habitat is more abundant than riffle habitat. Functional feeding group metrics are important to measure the trophic balance of the assemblage. However, misclassification of organisms to functional feeding groups may occur because life stage may alter the preferred feeding

mechanism (Maxted et al. in prep). Surrogate measures based on behavior or habit may provide a more robust measure. Such a metric based on the presence and composition of "clingers" has been found to a useful metric for the benthic assemblage (Fore et al. 1996, Maxted et al. in prep). We adopted percent clingers as a candidate metric to represent behavior or habit measures of the structure and function of the benthic assemblage.

Contribution of Chemical Contamination and Physical Habitat Degradation to Biological Impairment: Hypothesis Testing

The relationship of each benthic metric to habitat impairment and/or sediment contamination was evaluated by grouping sites by impairment categories (Table 4.4), and comparing the values of benthic metrics across categories using box & whisker plots (Fig. 4.4 and 4.5). This method allows comparison of the median, 25th and 75th percentile values, and the range between categories. In addition, *t-tests* of the significance of the difference between categories were also performed, and are displayed in the box & whisker plots. Linear regression of benthic metric values upon physical and chemical characteristics was also performed (Table 4.5). The hypotheses are supported if "Hab", "HabCon" and "Con" sites have poorer benthic metric values than "SomeHab" sites, if in turn "SomeHab" sites have poorer benthic metric values than "Ref" sites, and if benthic metric values are dependent upon total habitat scores and total toxic units scores (linear regression).

Table 4.4. Criteria for Site Categorization (see also Fig. 4.2 & 4.3)

Category	<u>Total Habitat Score</u>	<u>Total Toxic Units for Habitat Sample</u>	<u>Comment</u>
Ref (Reference)	155-185	0.5 - 1	outside watershed
SomeHab (Some Habitat Impairment; Horn Pond subwatershed sites)	97-120, (169)	0.5 - 3.5	Site H7 has a habitat score in the reference site range (169). It has a slightly higher Toxic Unit score (2). It was grouped geographically with the Horn Pond subwatershed sites.
Hab (Greatest Habitat Impairment)	60-81	1 - 2.5	
HabCon (Habitat Impairment and Contamination)	80-100	7 - 13	
Con (Greatest Contamination)	130-155	17 - 23	

Construction of an aggregate index

An aggregate index of the biological condition of macroinvertebrate assemblages was developed from the candidate benthic metrics (methods developed from Barbour *et al.* 1996b, Gibson *et al.* 1996). The requirements for inclusion of metrics in an aggregate index were the demonstrated ability to discriminate impaired from unimpaired conditions, non-redundancy with other metrics, and the inclusion of metrics from all four categories (taxa richness, composition, tolerance, feeding measures). One aspect of discriminatory ability is numerical: Barbour *et al.* (1996b) included in an aggregate index only those metrics with a median (for reference sites) of 6 taxa or more for taxa richness metrics and a median (for reference sites) of 15% or more for percentage metrics expected to decrease with impairment. These values were reasonable for the Boston Basin ecoregion. Using the four reference sites located outside of the Aberjona watershed to calculate median

reference values, we additionally required that percentage metrics that increase with impairment have a median (for reference sites) of less than 85%. (One metric was included that had a median of 5.5 for the reference sites.) The second aspect of discriminatory ability - sensitivity to impairment - was evaluated as described above: differences between metric values for reference sites and impaired sites were evaluated by comparing the median, the 25th and 75th percentile values, and the range between categories. Metric redundancy was evaluated using a Pearson Correlation matrix, and the results of bivariate scatterplots for metrics with correlations $r > 0.7$ (Barbour *et al.* 1996b). Metrics that met the criteria for inclusion in the aggregate macroinvertebrate index are referred to as core metrics.

Core metrics were normalized into unitless scores. The scoring concept is a modification of the methods of the Index of Biotic Integrity (e.g. Karr *et al.* 1986, Karr 1991). The 95th percentile value of metrics that decrease in response to perturbation was calculated. The region from 0 to the 95th percentile value was quadrisectioned and each region was scored 0, 2, 4, or 6, with high quality sites receiving the highest scores. The 5th percentile of metrics that increase in response to perturbation (e.g., % Hydropsychidae to Trichoptera, % Oligochaeta) was calculated. The region from the 5th percentile value to the maximum value observed across sites was quadrisectioned and each region was scored 0, 2, 4, or 6, with low values (indicative of high quality sites) receiving high scores. The aggregate index was the sum of the scores for each core metric.

The aggregate index should be a better measure of biological condition than any individual benthic metric because the aggregate index encompasses several aspects of the structure and function of the community (Barbour *et al.* 1996b). Thus, ideally, the relationship of the aggregate index (not the individual benthic metrics) to chemical and physical impairment would provide the best test of the hypotheses that: (a) in minimally contaminated aquatic environments of the Aberjona watershed, biological condition would vary from good to poor as habitat condition varied from good to poor, and (b) that in

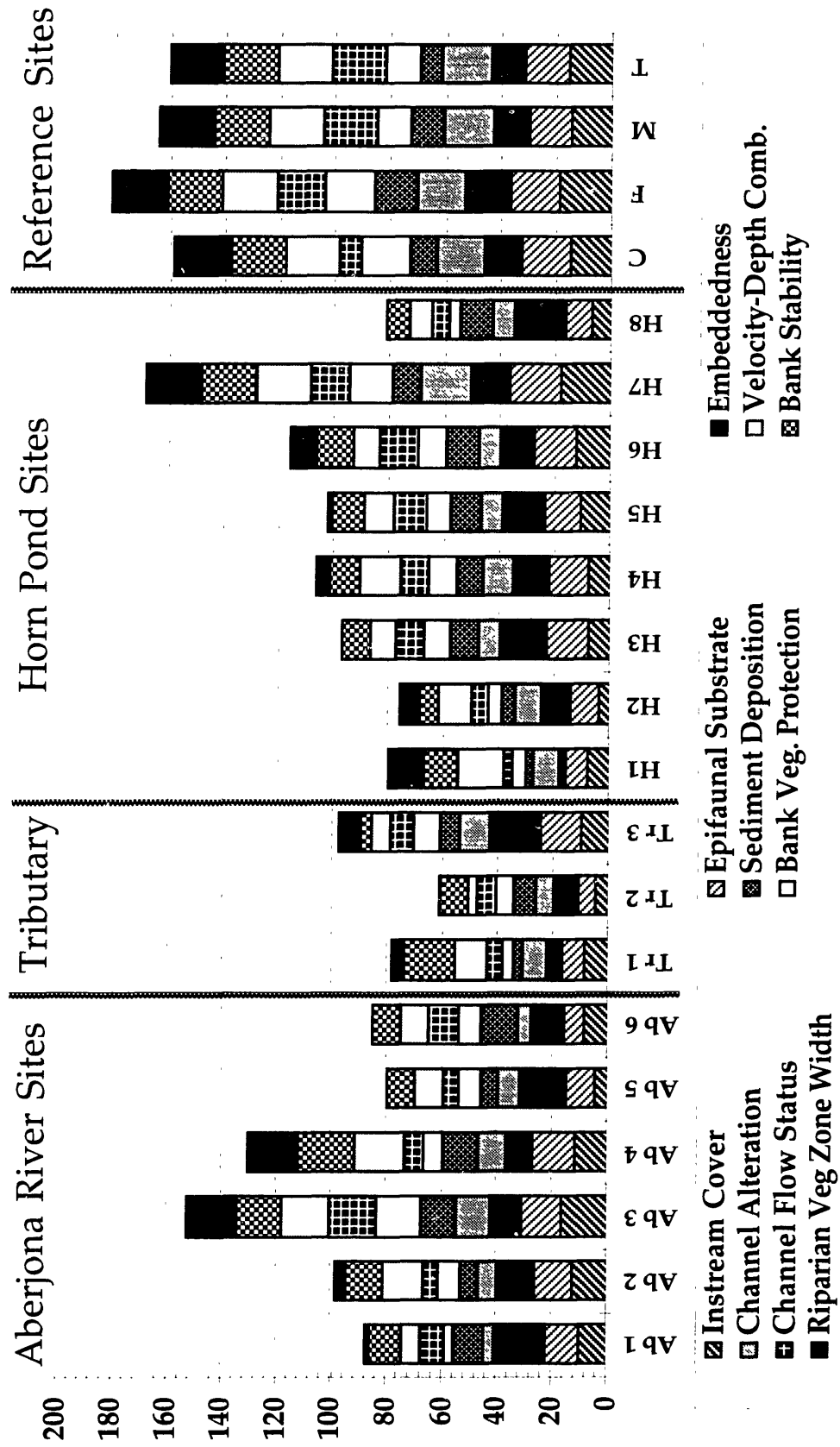
locations where concentrations of As, Cr, Cu, Pb and Zn exceeded ERM values, that biological condition would be degraded beyond expectations based upon habitat condition alone. The relationship of the aggregate index to total habitat score and total toxic units was evaluated, but caution is needed in the interpretation of that analysis because only those metrics with demonstrated sensitivity to habitat degradation and sediment contamination were included in the aggregate index.

Results

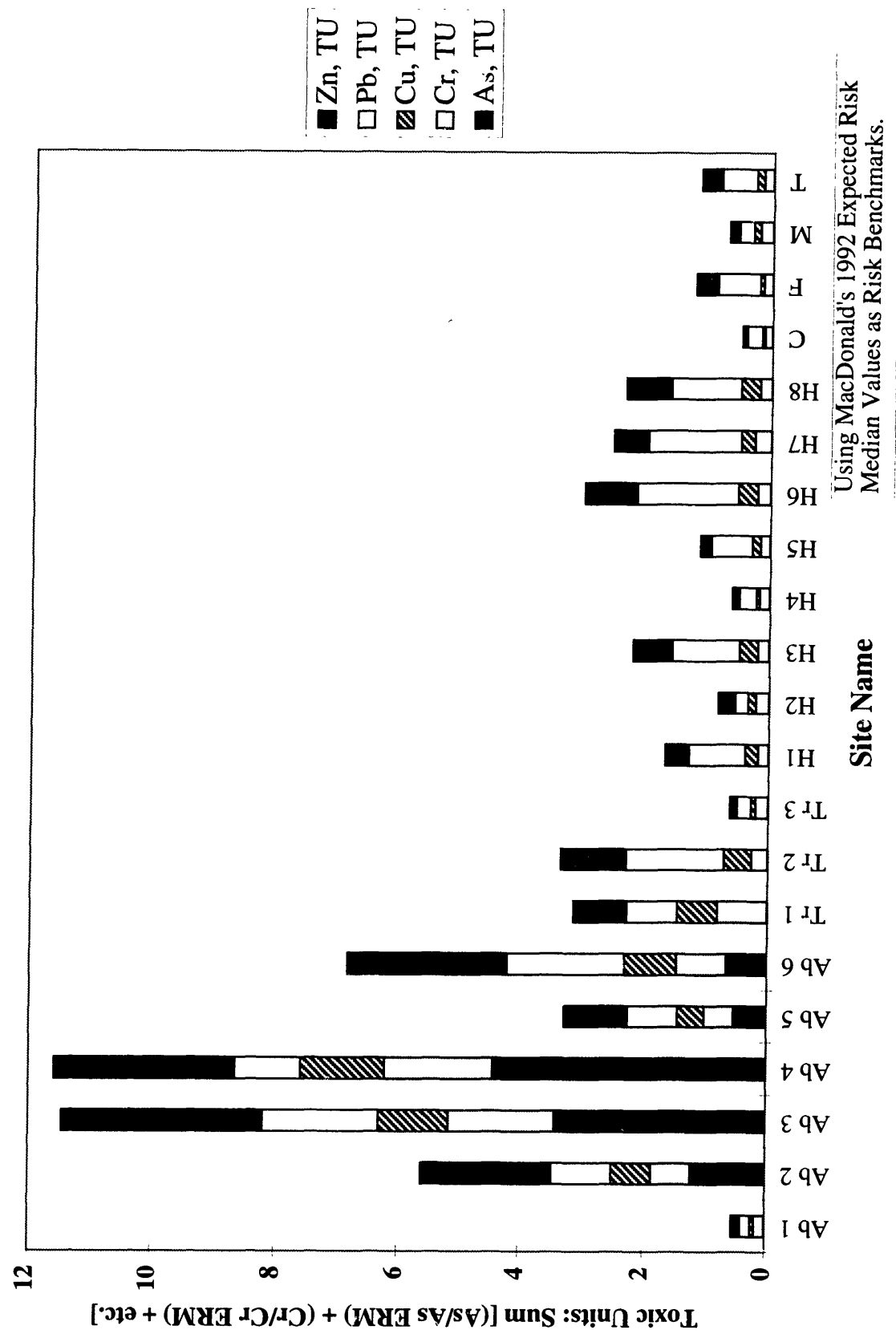
Characterization of Chemical and Physical Stress

“Ref” sites (C, F, M and T) had optimal habitat scores (Fig. 4.2) and minimal sediment (Fig. 4.3a) or epifaunal habitat (Fig. 4.3b) contamination by As, Cr, Cu, Pb or Zn. “SomeHab” sites (H3 - H7) had lower habitat scores (with the exception of site H7), and slightly higher concentrations of contaminants. “Hab” sites (Tr 1, Tr 2, H2 and H8) were severely habitat impaired, having total scores of only 60 to 81. “HabCon” sites (Ab 1, Ab 2, Ab 5 and Ab 6) were less habitat impaired and more contaminated than “Hab” sites. “Con” sites (Ab 3 and Ab 4) had intermediate habitat quality, but the highest concentrations of contaminants in the sediments and epifaunal habitat. Physical habitat scores followed the order: Ref > Con > SomeHab > HabCon > Hab. Thus, the application of the hypothesis that biological condition is largely determined by habitat condition in the absence of chemical stress becomes a hypothesis that the biological condition should follow the order: Ref > SomeHab > Hab. The application of the hypothesis that contamination impairs biological condition is then: Ref > Con, HabCon and SomeHab > HabCon.

Figure 4.2. Summed Habitat Scores



**Figure 4.3.a. As, Cr, Cu, Pb, Zn Sediment Contamination
Toxic Units vs. Sites**



**Figure 4.3.b. As, Cr, Cu, Pb, Zn Epifaunal Habitat Contamination
Toxic Units vs. Sites**

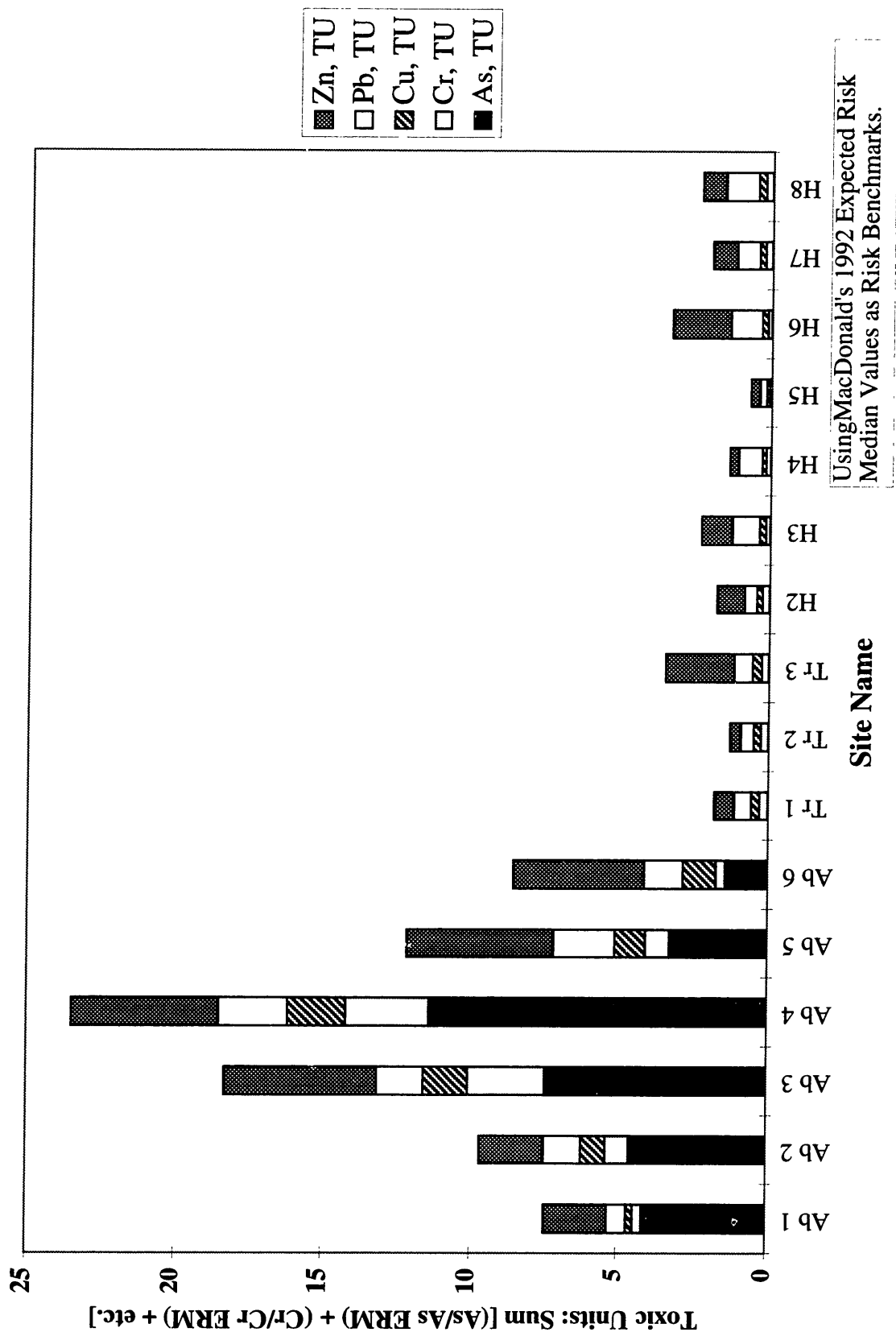


Table 4.5. Categorization of Sites

<u>Category</u>	<u>Sites Included</u>
Ref	C, F, M, T
SomeHab	H3, H4, H5, H6, H7
Hab	Tr 1, Tr 2, H2, H8 (H8 Dup)
HabCon	Ab 1, Ab 2, Ab 5, Ab 6
Con	Ab 3, (Ab 3 Dup), Ab 4

The four minimally-impaired reference sites received excellent scores for all physical habitat parameters except sediment deposition and embeddedness (Fig. 4.2). The lower scores for these two parameters are representative of the general condition of the Boston Basin ecoregion, and of the Aberjona watershed, in particular, which is characterized by slow-flowing streams with soft substrates. (Additional description of the habitat assessment results is included in Table 4.6.)

Concentrations of As, Cr, Cu, Pb and Zn in sediments from sites in the Aberjona watershed (Fig. 4.3) exceeded Long and Morgan's (1990, updated by MacDonald 1992) Expected Risk Low (ERL) and Expected Risk Median (ERM) values at many locations. Long and Morgan's criteria were developed for use by the National Status and Trends Program (NSTP) of the National Oceanic and Atmospheric Administration (NOAA) (Long and Morgan 1990, MacDonald 1992, Long and MacDonald 1992). The highest concentrations of As, Cr, Cu, Pb and Zn were found in epifaunal habitat samples, in which the concentrations were often double the concentration found in sediment samples.

Concentrations of Cr, Cu and Zn in the sediments of Horn Pond subwatershed and Tributary sites frequently exceeded ERL but not ERM values, indicating the possibility of adverse effects (Fig. 4.3). Arsenic concentrations at these sites were uniformly below the

Table 4.6 Habitat Assessment Results

<u>Parameter</u>	<u>Relevance of Parameter</u> (Tetra Tech, 1995)	<u>Discussion of Impacted Sites</u>
<u>Instream Cover</u>	Fallen logs and branches, large rocks, and undercut banks at reference sites provide fish with a large number of niches for feeding, laying eggs, and refugia. Without these niches, fish diversity decreases and the potential for recovery following disturbance decreases.	Instream cover scores at Horn Pond sites H2, H3, H4, and H5, and sites Ab 5 and Tr 2 were very low, making it difficult for these sites to support diverse fish assemblages. Sites H2, H3, H4 and Tr 2 were unlikely to support large fish assemblages anyway, because of their small size, but Ab 5 is part of the Aberjona River and its ability to support fish was probably greatly reduced by habitat impairment.
<u>Epifaunal Substrate</u>	Snags, submerged logs, and other hard substrates provide habitat for macroinvertebrates.	H2, H8, Tr 1, Tr 2, and Ab 5 and 6 had reduced ability to support macroinvertebrates.
<u>Embeddedness</u>	Embeddedness, caused by the deposition of fine sediments, reduces the availability of desirable fish and macroinvertebrate habitat.	Embeddedness severely reduced habitat availability at Tr 1; the stream bottom, all vegetation, and all rocks, snags and branches were coated in a thick layer of fine grained material (silt and mud). Tr 2's stream bottom consisted mostly of sand, fine pebbles and mud, thus it also had a low embeddedness score.
<u>Sediment Deposition</u>	Sediment deposition results from large-scale movement of sediment and creates an unstable environment for many organisms.	Fine grained materials can be deposited on macroinvertebrates, fish eggs and plants; this was probably adversely affecting organisms at Tr 1.
<u>Channel Alteration</u>	Channelization reduces natural habitat for fish, macroinvertebrates and plants and increases scouring.	Channelization was particularly severe at sites Tr 2, Ab 1, Ab 2, and Ab 6.
<u>Velocity-Depth Combinations</u>	Frequency of riffles, or variety of velocity-depth combinations, is a measure of a stream's ability to provide and maintain diverse and stable aquatic environment and its ability to handle surges in flow during storms.	Biological assemblages at sites H2, H8, Tr 1 and Ab 1 were probably impaired by the homogeneity of velocity and depth profiles at these sites.

Channel Flow Status	Channel flow status is important because it determines whether habitat (such as cobbles, logs, snags, overhanging bank vegetation and undercut bank roots) are submerged (available for aquatic fauna) or exposed to the air (unavailable).	Low scores for sites Tr 1, Tr 2, Tr 3, H2, H8, Ab 2, Ab 4, and Ab 5. Site H8 drains the entire Horn Pond subwatershed, so it should have had a greater flow than H7. Instead, the stream was shallow in October 1995 and August 1996, and was completely dry in large sections in August 1997. This was because flow was diverted from this site by pumping from aquifers upstream, in the Horn Pond area. As a result, during low flow conditions fish became stranded, and macroinvertebrate habitat was exposed.
Bank Vegetative Protection	Bank vegetation provides habitat (such as submerged overhanging bank vegetation) and organic inputs (leaves provide food for shredders and other macroinvertebrates) and its roots hold soil in place and prevent erosion.	Tr 2 had low scores for bank vegetative protection, bank stability, and riparian vegetative zone width.
Bank Stability	Eroded banks lack vegetative protection and contribute to sediment deposition.	Tr 3, H2 and H8 had the most serious problems with bank stability.
Riparian Vegetative Zone Width	Wide riparian zones provide a buffer for pollutants, control erosion, and provide habitat.	Riparian vegetative zone width scores were zero at five sites and below 10 for all sites except reference sites and Ab 3 and Ab 4.

detection limit of the XRF facility, which was approximately 20 ppm. The ERL for As is 8.2 ppm. Concentrations of Pb in sediments of the Horn Pond subwatershed and Tributary sites usually exceeded the ERL value, and sometimes exceeded the ERM value, indicating the likelihood of adverse effects. Concentrations of As, Cr, Cu and Zn were significantly higher in the sediments of many Aberjona River sites than in the sediments of other sites. Correspondingly, the exceedances (of ERL and ERM) are much greater.

Biological Condition and Contribution of Chemical Contamination and Physical Habitat Alteration to Biological Impairment

Three out of six of the general metrics (No. Total taxa, No. EPT taxa, % EPT; Fig. 4.4) had better values for “Ref” (reference sites C, F, M and T) than for “Hab” (habitat altered sites), “HabCon” (habitat altered and contaminated sites) and “Con” (contaminated sites). Comparisons of interquartile ranges and the use of *t-tests* indicated that these differences were significant (90% confidence level). The other three of the six metrics (HBI, % Dominant Taxon and % Filterers) did not show significant differences. The values of HBI were similar across categories. % Dominant Taxon and % Filterers were variable among the reference sites.

The supplemental benthic metrics (Fig. 4.5) also revealed differences between “Ref” and “Hab”, “HabCon” and “Con” sites. Significant differences (90% confidence level) were found between reference and impaired categories for five of the six supplemental metrics. % Orthocladiinae to chironomids varied greatly among the Ref sites, thus the metric was not useful for distinguishing impairment. The % Hydropsychidae/Trichoptera was significantly higher for all impairment types despite significant variability among reference sites. The number of scraper and piercer taxa was lower for all impairment types than for “Ref”.

Figure 4.4. General Benthic Metric Values for Each Site Category

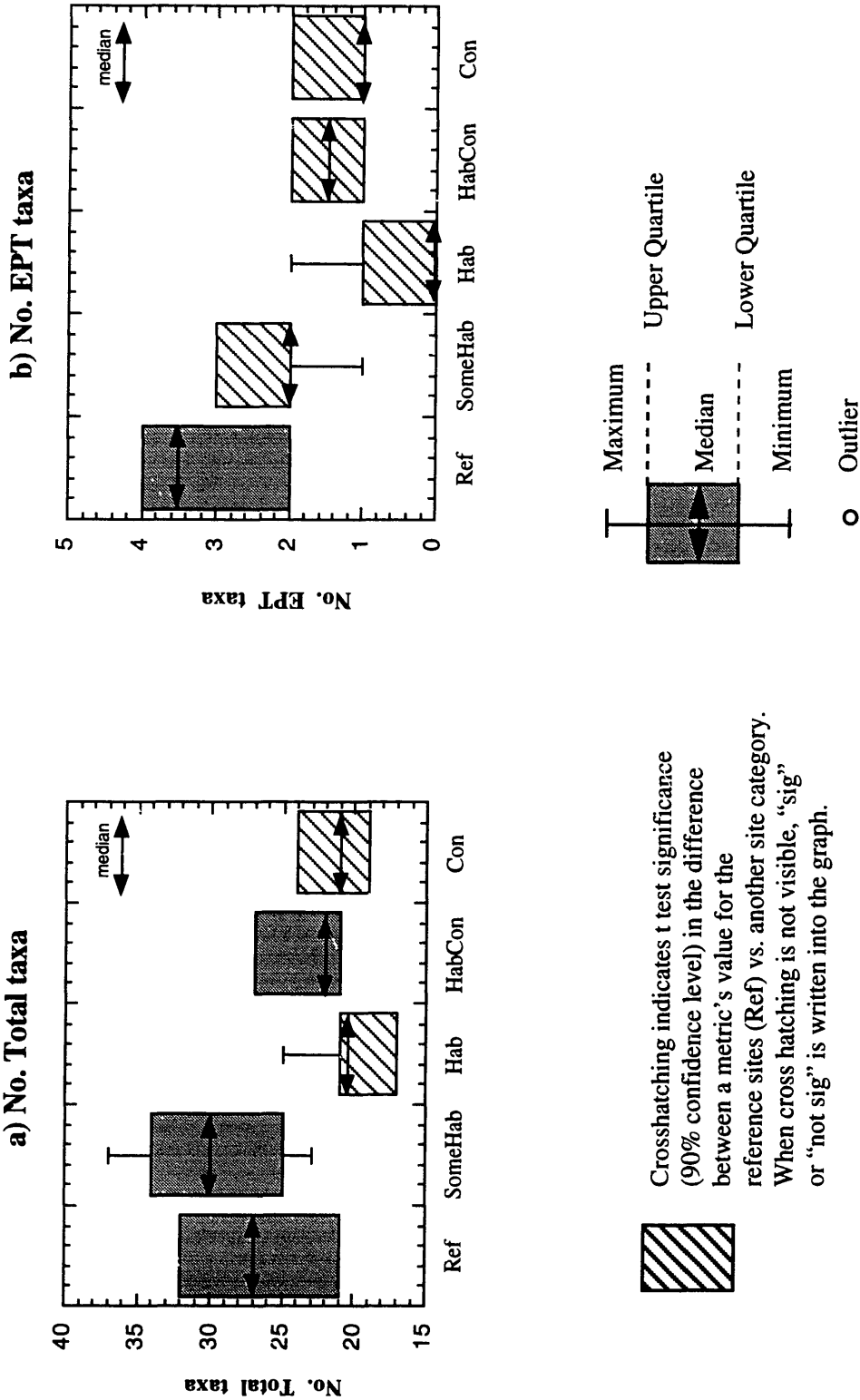


Figure 4.4. General Benthic Metric Values, Cont'd

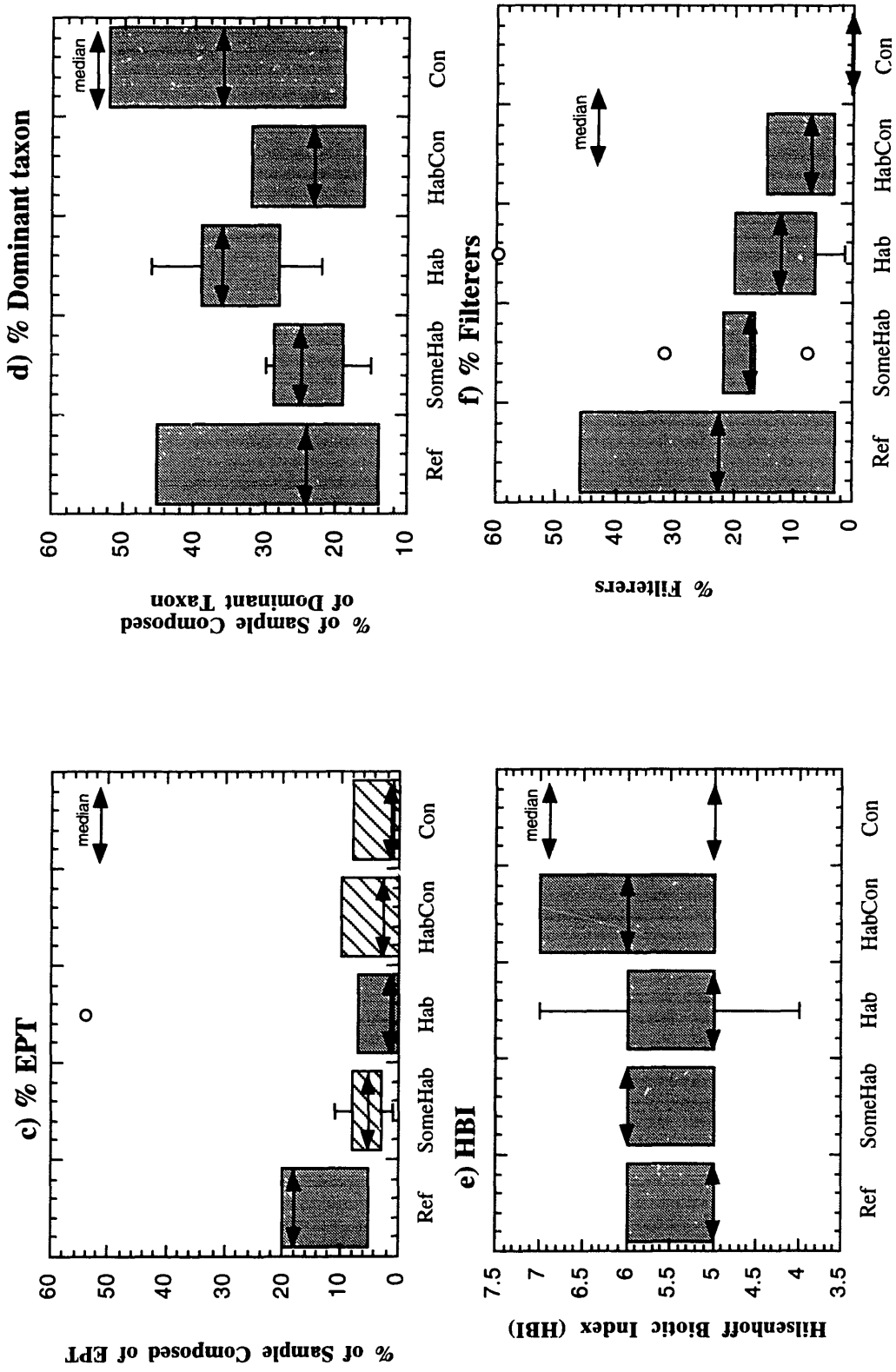
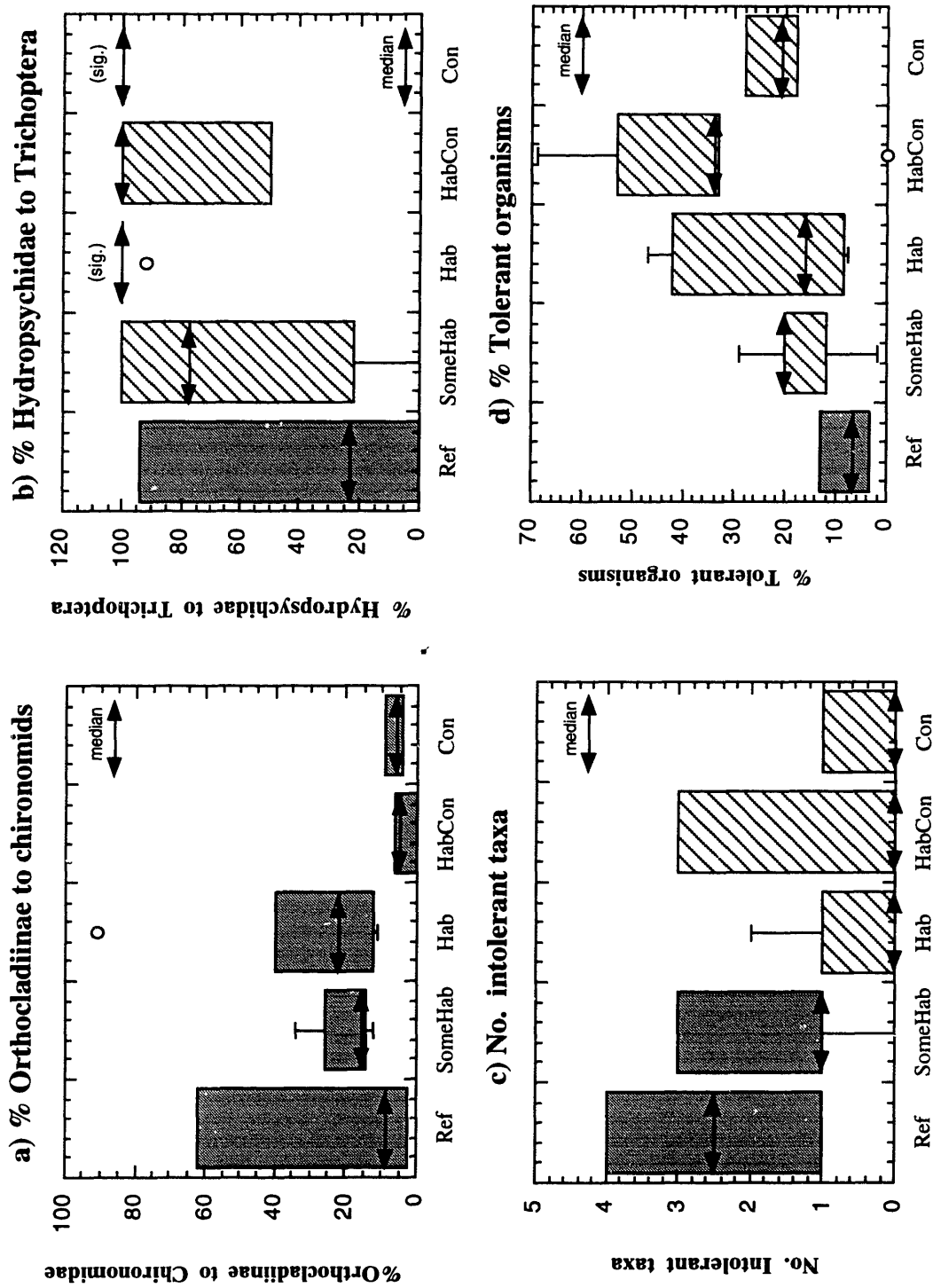


Figure 4.5. Supplemental Benthic Metric Values for Each Site Category



See Figure 4.4 for key.

Figure 4.5. Supplemental Benthic Metric Values, Cont'd

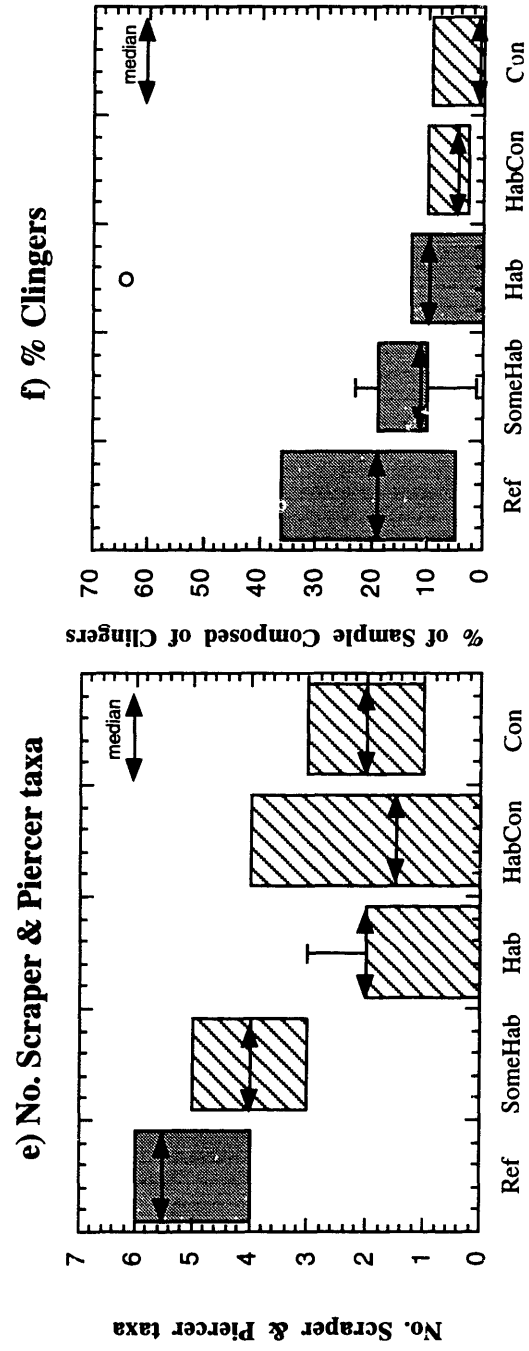


Table 4.7. Linear Regression Of Benthic Metrics Upon Total Habitat Scores And Total Toxic Units Measured in Epifaunal Habitat Samples.

Each of the core and supplemental metrics was linearly regressed upon total habitat score and the sum of the toxic units (for As, Cr, Cu, Pb and Zn in habitat samples). Significant dependence at the 90% confidence level is indicated by "yes". P-values for the regression are given in parentheses. Relationships between benthic metrics and explanatory variables (total habitat score and toxic units) were generally in the direction predicted (in Table 4.3); exceptions are listed in the table with strikethrough formatting. % Filterers can increase or decrease with impairment; % Orthocladiinae to chironomids was predicted to increase with metal contamination but did not.

Benthic Metric	Significant Dependence on Total Habitat Score? (P-Value)	Significant Dependence on Toxic Units (P-Value)	R-Square for regression
<u>General Metrics</u>			
Total No. taxa	no (.15)	no (.20)	.16
No. EPT taxa	yes (.05)	no (.26)	.21
% EPT	no (.64)	no (.30)	.06
% Dominant taxon	no (.80)	no (.40)	.04
Hilsenhoff Biotic Index	no (.28)	no (.63)	.07
% Filterers	no (.47) (direction variable)	no (.30) (direction variable)	.07
<u>Supplemental Metrics</u>			
% Orthocladiinae to chironomids	no (.18)	yes (.07) (.20 sediment)	.22
% Hydropsychidae to Trichoptera	yes (.08)	no (.16)	.21
No. Intolerant taxa	no (.14)	yes (.08) (.22 sediment)	.22
% Tolerant Organisms	yes (.01)	no (.15)	.34
No. Scraper & Piercer taxa	yes (.01)	yes (.08) (.06 sediment)	.36
% Clingers	no (.83)	no (.17)	.09
<u>Aggregate Index</u> (derived below)	yes (.005)	yes (.03) (.06 sediment)	.43

Four benthic metrics were significantly (90% confidence level) dependent upon total habitat score. The number of EPT (mayfly, stonefly, and caddisfly species) and Scraper & Piercer taxa were positively correlated with total habitat score (as predicted in Table 4.3). The percent Hydropsychidae to Trichoptera and the percent Tolerant Organisms were negatively correlated with total habitat score (as predicted in Table 4.3).

Nine metrics were not significantly dependent upon total toxic units measured in epifaunal habitat samples (regression results listed in Table 4.7); these metrics were also not significantly dependent upon toxic units measured in sediment samples. Three metrics (% Orthocladiinae to chironomids, No. Intolerant taxa, and Number of Scraper and Piercer taxa) were significantly dependent upon the total number of toxic units measured in epifaunal habitat samples, while only No. Scraper & Piercer taxa was also significantly dependent upon toxic units measured in sediment samples. Percent Orthocladiinae to chironomids was negatively correlated; this metric had been predicted to have a positive correlation.

Development of the Aggregate Index

Results of the selection process used to obtain the core metrics are listed in Table 4.8. *T-tests* (Figs. 4.4, 4.5) provided for the exclusion of four metrics because they were not significantly related to impairment. Three metrics failed the numerical discrimination test, leaving 6 remaining metrics (of an original set of 12). % Clingers was excluded because it was redundant with % EPT (Table 4.9). Thus, 5 metrics were used as core metrics and included in the aggregate index. Descriptive statistics for the core metrics and metric scoring is presented in Table 4.10.

Table 4.8. Selection of Core Metrics: Summary Table					
Metric	Sensitive to Impairment ^a	Median (4 non-Aberjona reference sites)	Numerical discrimination possible	Redundant ^b	Core Metric
No. Total taxa	yes	27.0	yes	no	yes
No. EPT taxa	yes	3.5	no	*	
% EPT	yes	18.3	yes	no	yes
% Dominant Taxon	no	24.0	yes	*	
Hilsenhoff Biotic Index	no	5.0	yes	*	
% Filterers	no	22.8	yes	*	
% Orthoclaadiinae to chironomids	no	8.8	no	*	
% Hydropsychidae to Trichoptera	yes	23.1	yes	no	yes
No. Intolerant taxa	yes	2.5	no	*	
% Tolerant Organisms	yes	6.8	yes	no	yes
No. Scraper & Piercer	yes	5.5	marginal	no	yes
% Clingers	yes	19.0	yes	with %EPT	
^a Determined with <i>t</i> -tests (Fig. 4.5)					
^b Determined with Pearson Product Correlation (Table 9)					

Table 4.9. Pearson Correlation Matrix of Benthic Metrics.

	No. Total taxa	% EPT	% Hydropsychidae to Trichoptera	% Tolerant Organisms	No. Scraper & Piercer	% Clingers
No. Total taxa	1.00					
% EPT	-0.04	1.00				
% Hydropsychidae to Trichoptera	-0.32	-0.21	1.00			
% Tolerant Organisms	-0.32	-0.41	0.37	1.00		
No. Scraper & Piercer	0.60	0.29	-0.63	-0.67	1.00	
% Clingers	0.05	0.94	-0.21	-0.50	0.40	1.00

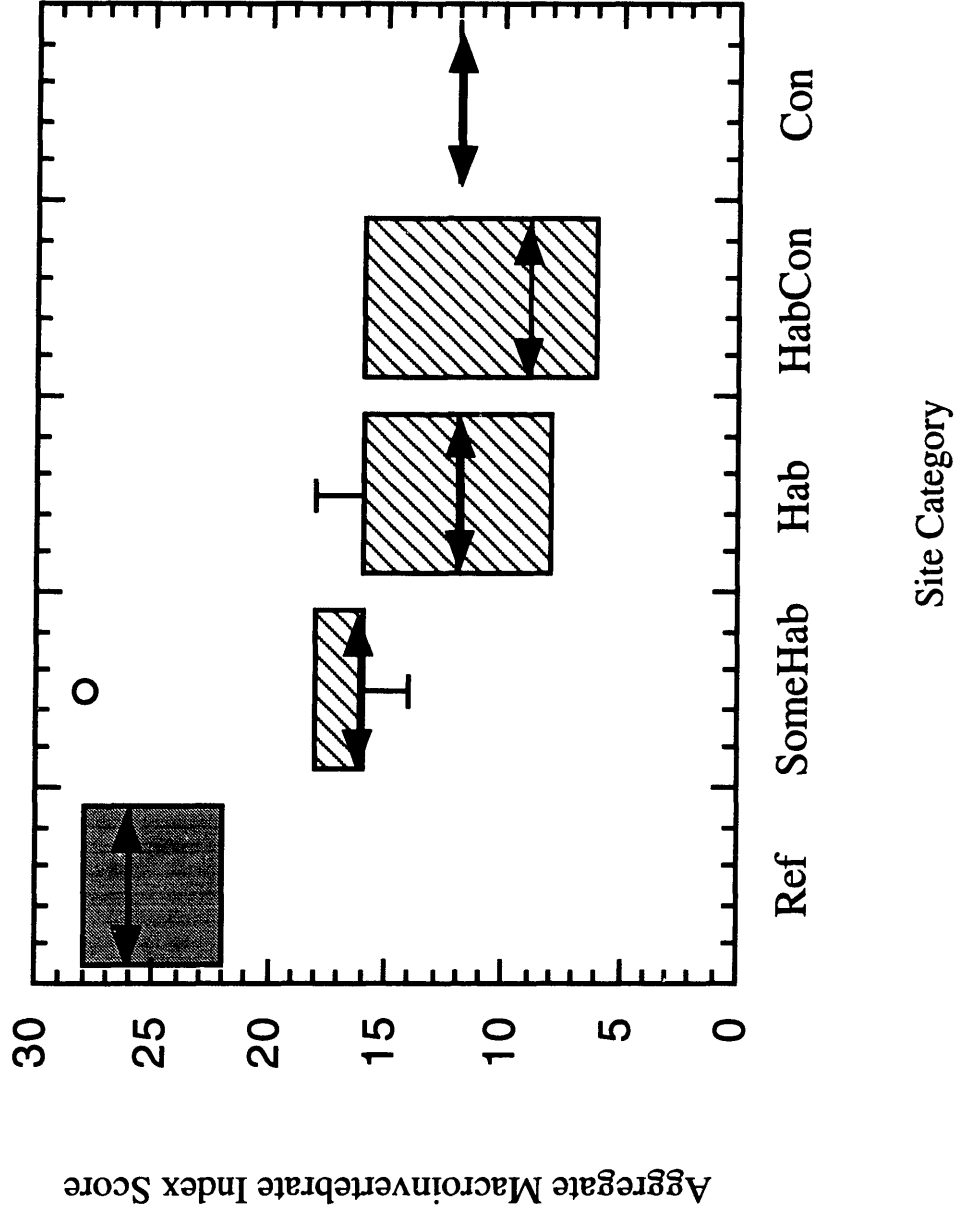
Table 4.10. Descriptive statistics and scores for the core metrics. (Reference Barbour <i>et al.</i> 1996b)										
Metric	Statistics						Score			
	min	5%	50%	95%	max		6	4	2	0
No. Total taxa	15.0	17.2	23.0	33.8	37.0		> 25	17-25	9-16	< 9
% EPT	0.0	0.0	3.7	20.9	54.3		> 16%	10-16%	5-9%	< 5%
% Tolerant taxa	2.0	3.5	20.3	53.9	69.1		< 24%	24-47%	48-72%	> 72%
% Hydropsychidae/Trichoptera	0.0	2.2	100.0	100.0	100.0		< 24%	24-47%	47-73%	> 73%
No. Scraper & Piercer taxa	0.0	0.0	3.0	5.9	6.0		> 4	3-4	2	0-1

The resulting aggregate index is presented in Figures 4.6 and 4.7. Impaired categories received significantly (*t-tests*, 90% confidence level) lower aggregate index scores than Ref sites. SomeHab sites received scores in the higher range of the impaired categories. Hab, HabCon and Con had index values in a similar range (Fig. 4.6). Duplicate macroinvertebrate samples for sites Ab 3, H8 and Tr 3 received similar aggregate macroinvertebrate index scores as the original samples (Fig. 4.7), yielding confidence in the methods. Aberjona River sites received poor or very poor ratings. Most sites in the Horn Pond subwatershed received poor ratings, although two received the minimum value for good ratings, and one received a very good rating. Tributary sites all received very poor ratings, and Ref sites received good or very good ratings.

Discussion

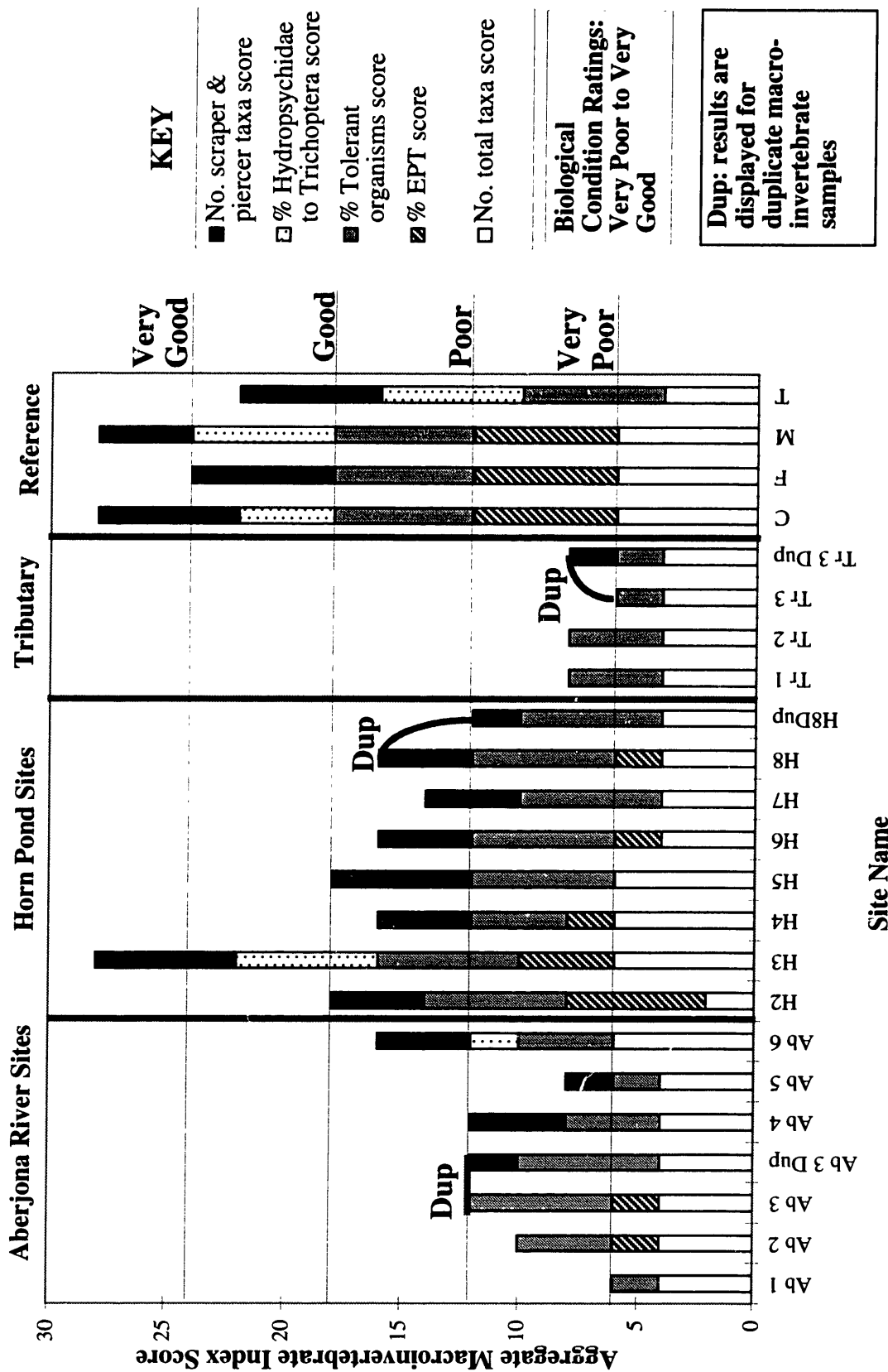
We believe that the epifaunal macroinvertebrate assemblages that are sampled to assess the biological condition of low-gradient streams (i.e., streams whose habitats are not dominated by riffles) have greater exposure to contaminants in the epifaunal habitats sampled in this study than to contaminants in the fine-grained sediments that are traditionally collected in ecological risk assessments. Thus, we find it interesting that: (a) the highest concentrations of As, Cr, Cu, Pb and Zn, and thus the highest toxic ratios (toxic ratio = concentration / ERM), were measured in epifaunal habitat samples, not in sediment samples (Fig. 4.3a vs. 4.3b), (b) the concentrations of these elements in epifaunal habitat samples were often twice as high as the concentrations found in the sediment samples, and (c) the ranking of sites according to contamination level depended upon which sample type (sediments or epifaunal habitat) was used. The difference between the toxic ratios for Aberjona River samples and the toxic ratios for Horn Pond and Tributary site samples was greater for epifaunal habitat samples than for sediment samples. In the case of site Ab 1, the concentrations of contaminants were low in the sediment sample, but high in the epifaunal habitat sample.

Figure 4.6. Distribution of Aggregate Macroinvertebrate Index Scores For Each Site Category



See Figure 4.4 for key.

**Figure 4.7. Aggregate Macroinvertebrate Index Score:
Summed Benthic Metric Scores For Each Site**



As predicted, benthic metric values for sites with chemical and physical degradation were poorer or indistinguishably different from benthic metric values for reference sites. Severe physical degradation, moderate chemical and physical degradation, and severe chemical degradation were associated with similar levels of biological impairment. None of the benthic metrics received better scores for Hab (severely habitat impaired) sites than for Ref (non-Aberjona watershed reference) sites. Six benthic metrics had significantly (90% confidence level, *t-tests*) better scores for Ref than Hab sites, and 5 benthic metrics had significantly better scores for Ref sites than SomeHab (habitat quality intermediate between Ref and Hab sites). In addition, linear regression indicated that 4 metrics were significantly dependent (90% confidence level, linear regression) upon total habitat score. Although the other 8 metrics were not significantly dependent upon total habitat score, 7 responded in the direction predicted and 1 did not have a predicted direction. Together, the results provided strong evidence that physical habitat degradation in the Aberjona watershed was adversely impacting macroinvertebrate assemblages.

The biological impairment associated with physically degraded sites appears to be related to the urbanization of the Aberjona watershed. Urbanization has impaired the physical integrity of the Aberjona watershed in a number of ways (Table 4.6) that have decreased the quantity and quality of habitat for aquatic macroinvertebrate assemblages. As the land has been developed for residential, commercial, and industrial uses, stream channels and the land beside them (the riparian zone) have been altered. Stream channels have been straightened, deepened, or diverted into artificial (e.g., concrete or metal) channels. Urban development alters flow regimes (Karr *et al.* 1986), and probably explains the low channel flow status observed at many sites in the Aberjona watershed. Rain falling on a vegetated surface can be absorbed into the ground and slowly released to streams. When rain falls on paved surfaces in urban areas, it cannot be absorbed, thus stream flow can be sharply increased during storms and greatly decreased during dry periods. In addition to this general effect of urbanization, stream flow has been severely

reduced downstream of Horn Pond by groundwater wells pumping from an aquifer hydrologically connected to Horn Pond. The effect of pumping has been to reduce stream flow so drastically that site H8, which is located downstream of Horn Pond, had a measured stream depth of 20 cm in October 1995 and August 1996 and was completely dry in several sections in August 1997.

The evaluation of chemical degradation followed the approach of the National Status and Trend Program (NSTP). The NSTP approach for evaluating contaminated sediments presumes that the potential for toxicity is relatively high in areas where numerous chemicals exceed the ERM, and that the potential for toxicity is low in areas where none of the chemical concentrations exceed the ERL. Recommended uses of the guidelines (Long and MacDonald 1992) include ranking and prioritization of areas and sampling sites for further investigation, quantification of the relative likelihood of toxicity over specific ranges of chemical concentrations, and assessment of potential ecological hazards of contaminated sediments. The NSTP approach indicated that the greatest ecological hazard existed at sites Ab 3 and Ab 4, and that great hazard also existed at sites Ab 2, Ab 5 and Ab 6. The NSTP approach is based upon the evaluation of contaminated sediments, and was also adopted on a trial basis in this study to evaluate contaminated epifaunal habitats.

Biological impairment was observed at sites where ecological hazards were identified by NSTP approach. Eight benthic metrics had significantly (90% confidence level, *t-tests*) better scores for Ref than Con sites, and 7 benthic metrics had significantly better scores for Ref sites than HabCon sites. In addition, of the 8 benthic metrics with better scores for Ref than Con sites, 7 of these had better median values for SomeHab sites than for Con sites, even though Con sites had higher total habitat scores than SomeRef sites.

Benthic metrics were not diagnostic of impairment type: no benthic metric had optimal values for Hab and impaired values for Con, or vice versa. Percent Orthocladiinae to chironomids was selected as a supplemental metric because it has been found to be

positively correlated with contamination by As, Cr, Cu, Pb and Zn in several descriptive studies and to Cu and Zn in experimental studies of water pollution (Clements 1991). No significant differences between categories were found, but it was surprising that the median value for this metric was lower for the two contaminated site categories (HabCon, Con) than for the somewhat and severely habitat impaired categories (SomeHab and Hab). Linear regression provided weak support for the hypothesized impact of metals upon macroinvertebrate assemblages. The number of intolerant taxa and the number of scraper and piercer taxa were significantly dependent upon total toxic units in epifaunal habitat samples.

The aggregate macroinvertebrate index developed in this paper illustrated the strength of the multimetric index approach for detecting the cumulative impacts of physical habitat degradation and chemical contamination. While subselection of metrics that were sensitive to chemical and/or physical impairment necessarily resulted in an aggregate index that was more sensitive to impairment than the metrics on average, the aggregate index was *more* sensitive to impairment (as measured by linear regression on total habitat and toxic units scores) than *any* individual metric. Of the five metrics that compose the aggregate index, only one was individually significantly (90% confidence level) dependent upon total toxic units score and only three were individually significantly (90% confidence level) dependent upon total habitat score. The aggregate index was significantly dependent upon total habitat score at the 99% confidence level, and upon the total toxic units score at the 95% confidence level.

The duplicate macroinvertebrate samples (sampled at the same time and location as the original samples) received aggregate index scores that differed from the scores received by the original samples by at most 4 points (the differences for the three samples were 0, 2 and 4 points). The ratings (i.e., very poor, poor, good, or very good) assigned to each of these duplicate samples were identical to the ratings assigned to the original samples.

Aggregate index scores (based solely on biological information) yielded ratings that were consonant with the classifications assigned on the basis of chemical and physical information. Ref sites received good or very good ratings; SomeHab sites received poor, good or very good ratings; Hab, HabCon and Con sites received poor or very poor ratings.

Conclusions

This paper contributes to the development of assessment methods for urban watersheds. Methods were developed to select appropriate ecoregional reference sites for urban systems and to use impairment gradients to examine the individual and cumulative impacts of chemical and physical stressors. The classification of sites into categories (e.g., reference sites, habitat degraded sites, habitat degraded & contaminated sites, contaminated sites) was accomplished using multimetric indices of physical habitat condition (total habitat score is the sum of the scores for 10 habitat parameters) and contamination (total toxic units score is the sum of the ratios of As, Cr, Cu, Pb and Zn concentrations to their respective ERM values). The sampling of epifaunal habitats provided a measure of exposure of macroinvertebrates to contaminants that, to our knowledge, has not been used before. Benthic metrics that satisfied the criteria of sensitivity to impairment, numerical discriminatory ability, non-redundancy, and inclusion of the four categories of metrics (taxa richness, composition, tolerance/intolerance, and feeding measures) were incorporated into an aggregate macroinvertebrate index of the biological condition. The aggregate macroinvertebrate index needs to be tested on new sites for which it was not developed. It would be especially useful to analyze the response of the aggregate index to other stressors not addressed in this study (e.g., eutrophication, dramatic alterations to stream flow). The appropriateness of the aggregate macroinvertebrate index is expected to vary regionally. The methods described in this paper could be used to develop macroinvertebrate indices for urban systems in other regions.

The assessment methods described in this paper have several applications. State environmental protection agencies may find the assessment methods useful for a variety of program applications, many of which have not yet been implemented for urban watersheds because of the difficulties addressed in this paper. Examples of environmental protection program applications were described by Gibson *et al.* (1996, p. 133) as purposes served by biocriteria: to help (1) characterize the condition of aquatic resources, (2) refine aquatic life use categories, (3) judge use impairment (i.e., to help determine attainment and non-attainment of designated uses), (4) identify possible sources of impairment (e.g., habitat degradation or biological imbalance), (5) screen for problems, (6) rank and establish priorities of surface waters for needed remedial actions, and (7) assess the results of new management practices imposed to mitigate problems.

The assessment methods also have applications for field studies of contaminant impacts upon macroinvertebrate assemblages. Because the methods directly contribute to the understanding of the biological condition and the relationship of biological impairment to chemical contamination and physical habitat degradation, they can be used in ecological risk assessments, contaminant impact research, and the development of sediment quality criteria. Ecological risk assessments involve the identification of assessment endpoints, characterization of exposure to stressors and the effects of stressors, and risk characterization. Benthic metrics and aggregate macroinvertebrate indices could aid in the development of assessment endpoints, sampling of epifaunal habitats might be included in the characterization of exposure to stressors, and the methods used to relate stressors to biological impairment could aid in the characterization of the effects of stressors and the final characterization of risk. Field studies of the impacts of contaminants upon macroinvertebrate assemblages have been hindered by the difficulties involved in identifying the impacts of contaminants in environments with multiple stressors. This paper addressed those difficulties and assessed multiple stressors individually and cumulatively.

The methods described in this paper could be used to improve the database used to calculate ERM values. The ERM values used to calculate total toxic units for sediment and epifaunal habitat samples were developed using the Biological Effects Database for Sediments (BEDS) (Long and MacDonald 1992). The data included in the BEDS were compiled from numerous modeling, laboratory and field studies. The method used to evaluate adverse contaminant effects in field studies could be improved, possibly resulting in ERM values that are more predictive of field effects. The method that was used (Long and MacDonald 1992) assumed that macroinvertebrate assemblages were adversely affected by contaminants if a field study included sites with low concentrations of contaminants with robust communities and sites with contaminant concentrations that exceeded background, reference or non-affected samples by two-fold or more and had relatively depauperate benthic communities (i.e., those with low abundance or species richness). A multimetric index such as the one described in this paper would probably provide a better measure of biological condition than abundance or species richness alone. The methods used in this paper to relate biological impairment to physical habitat degradation and chemical contamination of the benthic environment (sediments and epifaunal habitats) could probably attribute biological impairment to chemical contamination more accurately than the method used for the BEDS. If the methods described in this paper were supplemented with toxicity testing and/or consideration of other stressors, further gains in accuracy could be expected.

While improvements to the BEDS database might be achieved, it is encouraging that the NSTP (National Status and Trends Program) approach appeared to work so well in this study. The sites with high total toxic units (in the epifaunal habitat samples, and to a lesser extent, in the sediments for which the NSTP approach was designed) were identified by the NSTP approach as sites with a high probability of being impacted by contaminants; the analyses presented in this paper suggested that biological impairment at these sites could be attributed to contamination.

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Chapter 5:

Conclusions and Management Implications

A method to assess the ecological integrity of an urban watershed was developed that has the potential to contribute to the protection and restoration of urban streams (Table 5.1). I suggest that a plan for watershed restoration should begin with a preliminary descriptive assessment of chemical, physical and biological conditions that seeks to identify causal relationships between observed chemical and physical degradation and biological impairment. Remedial actions can be designed based upon the preliminary assessment. (For example, remedial alternatives for the Aberjona watershed are described and discussed in Table 5.2.) Continued monitoring of the ecosystem's response to remediation, then, provides an opportunity to move from descriptive assessments that can only hypothesize about relationships between variables to more experimental frameworks in which some variables are changed and the responses of other variables are monitored.

Table 5.1. Aspects Of The Dissertation That Contribute To Environmental Management

Aspect Of The Dissertation	Contribution To Environmental Management
The Method Developed For The Dissertation Can Be Used To ...	Conduct A Preliminary Descriptive Assessment To Identify Threats To Ecological Integrity
	Conduct Continued Monitoring To Evaluate The Effectiveness of Restoration Efforts
The Aggregate Macroinvertebrate Index Can Be Used To ...	Characterize The Condition Of Aquatic Resources
	Refine Aquatic Life Use Categories
	Judge Use Impairment
	Screen For Problems / Rank and Establish Priorities Of Surface Waters For Needed Remedial Actions
	Aid In The Development Of Assessment Endpoints For Ecological Risk Assessment

The Sampling Of Epifaunal Habitats Could ...	Aid In The Characterization Of Exposure To Contaminants (e.g., Ecological Risk Assessment)
The Methods Developed To Relate Stressors To Biological Impairment Could ...	Aid In The Characterization Of The Effects Of Stressors Upon Biological Assemblages (Ecological Risk Assessment)
	Aid In The Characterization Of Overall Risk (Ecological Risk Assessment)
The Inclusion Of Physical Habitat Assessment And Methods To Assess The Cumulative Impacts Of Chemical And Physical Stressors Leads To ...	Improved Accuracy For Field Investigations Of Contaminant Impacts (A Variety Of Applications, Including Improvement Of The Biological Effects Database For Sediments, Used To Calculate Expected Risk Median Sediment Quality Benchmarks)
The Assessment Of The Aberjona Watershed Provided a ...	Foundation For A Remedial Strategy For The Aberjona Watershed
The Attribution Of Biological Impairment To Physical Habitat Degradation Suggests That ...	The Restoration Of Biological Integrity Depends Upon Restoration And Maintenance Of Riparian Habitat
The Difficulty Encountered In Locating Suitable Reference Sites For The Boston Basin Suggests That...	The Problem Of Physical Habitat Degradation Is Widespread In The Boston Basin Ecoregion

Foundation for a Remedial Strategy for the Aberjona Watershed

It was not the purpose of this dissertation to design a comprehensive plan to restore the Aberjona watershed. Such a plan would require many conversations with watershed stakeholders, and in-depth analysis of the social, economic and environmental costs and benefits of various remedial options. This section explores remedial actions that could address problems identified by the dissertation, and includes some discussion of alternatives.

Table 5.2. Discussion of Remedial Alternatives for the Aberjona watershed

Remedial Action	Discussion
Reduce the flow of contaminants from the Industri-Plex 128 Superfund site into the Halls Brook Storage Area (HBSA), and prevent the migration of contaminated sediments from the HBSA to the Aberjona River.	Reducing the continuing groundwater inputs of toxic chemicals to the HBSA could reduce the risks posed to fish and macroinvertebrates living in and downstream of the HBSA.
Sediments could be dredged from the Halls Brook Storage Area (HBSA) and the Aberjona River, and the contaminated peat deposits of the Wells G and H wetland could be removed.	While this would address the contamination problem, such a large dredging operation would be very expensive, and (at least in the short term) would destroy aquatic habitat on a massive scale and disturb fish and macroinvertebrate assemblages.
Re-engineer stream channels to their original shape, remove all buildings from the riparian zone, and revegetate the entire area.	This remedial action could greatly improve physical habitat quality, but would result in a level of expense and social dislocation that is unlikely to be perceived as acceptable.
Revegetate the stream banks with trees, shrubs, and other plants.	This remedial action could improve habitat quality in a number of ways with relatively low social and economic costs. (See text.)
Use porous materials when designing new paved surfaces or repairing old ones.	Urban areas tend to be dominated by impervious surfaces such as roads, parking lots and buildings. The use of porous pavement would result in less disruption of the natural hydrologic cycle.
Add vegetation to the landscape and designate land for parks to increase the land's ability to absorb rainfall for slow release to streams.	This remedial option also helps to restore the natural hydrologic cycle. Enhanced aesthetic qualities and increased terrestrial habitat are added benefits of this remedial action.

Construct wet or dry stormwater retention basins.	When large quantities of water rush across paved surfaces, sediments, oil, road salt and other pollutants are often carried along with the water and end up in the streams. Stormwater retention basins can reduce the impacts of urban stormwater on streams.
Re-engineer road crossings.	In many areas of the Aberjona watershed, channels have been built at stream crossings that send water directly from the roads to the streams. Other construction options are available that offer greater protection to streams.
Reduce the quantity of water withdrawn from the municipal wells that pump groundwater from the aquifer connected to Horn Pond.	<p>Fish and macroinvertebrate assemblages downstream of Horn Pond appeared to be severely impacted by loss of stream flow. Great improvements in biological condition would probably result from reducing this diversion of water.</p> <p>The water that is withdrawn is used by the City of Woburn for a variety of human purposes, including residential drinking water. It is probably unrealistic to expect the town of Woburn to eliminate the use of the pumps, given their importance. Instead, water conservation efforts could be encouraged, especially during dry months.</p> <p>(Sampling was performed in October 1995 and August 1996, months when the impacts of water diversion were probably particularly severe. Additional monitoring efforts could aid in determining the seasonality of impact.)</p>

Revegetating the stream banks with trees, shrubs, and other plants would increase physical habitat quality in a number of ways that would be reflected by increased scores for several physical habitat parameters (Appendix A). Instream cover and epifaunal substrate scores would increase because a revegetated riparian zone would provide new habitat in the form of fallen trees and branches, overhanging bank vegetation and undercut bank roots. Embeddedness and sediment deposition scores might increase due to the enhanced ability of the revegetated banks to catch sediments before they enter the stream, and the decreased erosion potential of the banks. (Plant roots help to hold the soil in place.) The variety of velocity-depth combinations might increase if slow-moving deepwater habitats were formed behind fallen trees and logs. Bank vegetative protection would be increased directly. Bank stability would be enhanced by plant roots, and the riparian vegetative zone width would be increased in many areas from almost zero to the width of the newly vegetated zone.

An effective restoration plan for the Aberjona watershed will need to address both chemical and physical degradation. Significant biological impairment appears to be related to chemical contamination and habitat degradation, and in areas suffering from both problems, remediation of only one problem is unlikely to produce large ecological benefits. Selective contaminant removal combined with habitat restoration measures could yield significant benefits. Stream bank revegetation, improved stormwater management and water conservation measures could improve the physical quality of the Aberjona watershed's aquatic habitats. Additional problems that would need to be addressed as part of a comprehensive watershed restoration plan would include eutrophication, sewage overflows, improper dumping of household chemicals and car fluids, and excessive use of pesticides; these problems were not specifically addressed by the dissertation.

The Resource Requirements Of The Method Are Modest; It Is Realistic To Suggest The Use Of The Method By Environmental Protection Agencies

The method (described in parts in chapters 2, 3 and 4, and listed as a whole in Appendix D) is fully available to state environmental protection agencies and has modest resource requirements. Physical habitat assessment, sediment sampling, epifaunal habitat sampling, and macroinvertebrate sampling could be performed in a single visit by one senior and one junior investigator. Fish sampling could be performed on a separate occasion by one senior investigator and two junior investigators. (Performing fish and macroinvertebrate sampling during the same visit is not recommended because walking through the stream to sample the first assemblage disturbs the second assemblage.) Because flow conditions vary widely, the fish sampling team should also measure stream width and depth. Many sites could be visited by each team in a single day, depending on distance between sites, the difficulties of accessing the site, and site conditions. Sampling could occur on a yearly or more frequent basis. In this dissertation, physical habitat was assessed and macroinvertebrates were sampled according to the protocols used by the Massachusetts Department of Environmental Protection (MA DEP) (Tetra Tech, 1995). The MA DEP's protocol for fish assemblage assessment (Tetra Tech, 1995) could replace the method used here. The MA DEP's method is somewhat more resource-intensive, but yields higher quality data. Some environmental protection agencies might not have the necessary equipment for X-Ray Fluorescence analysis (used to analyze metal concentrations in sediments and epifaunal habitats) or Instrumental Neutron Activation Analysis (used to measure metal concentrations in fish). In these cases, other analytical techniques could be used. Data analysis and interpretation was accomplished using standard spreadsheet and word processing software (Microsoft Word, Excel and Powerpoint, and KaleidaGraph) already in use in most agencies.

Conclusions

The ecological assessment of the Aberjona watershed involved developing a reference condition for the Boston Basin ecoregion, a sampling method to measure the exposure of biological assemblages to epifaunal habitats, an aggregate macroinvertebrate index of biological condition, and methods to relate biological impairment to the cumulative effects of chemical and physical stressors. The potential for various aspects of the dissertation to contribute to environmental management are numerous (Table 5.1).

This dissertation has emphasized the significance of physical habitat degradation as a threat to biological integrity, and the importance of integrating chemical, physical and biological assessment. This message has particular relevance for environmental protection agencies and ecological risk assessors. The Clean Water Act's objective "to restore and maintain the chemical, physical and biological integrity of the Nation's waters" presents a formidable challenge to environmental protection agencies. Typically, different divisions of the same agency, or different divisions of different agencies, are responsible for hazardous waste sites, monitoring water quality, issuing pollution discharge permits, addressing nonpoint source urban and agricultural pollution, wetlands, protecting endangered species, and handling zoning permits. While there are good reasons to partition responsibilities, this partitioning can present an obstacle to integrated environmental protection and assessment. Similarly, the current practice of assessing contaminant risks with ecological risk assessments that do not consider the physical risks of habitat degradation limits the effectiveness of risk assessments. The risk assessor may not be able to distinguish contaminant impacts from the impacts of physical habitat degradation, and the opportunity to put contaminant risks in context with other risks is lost. The usefulness of ecological risk assessments and the effectiveness of environmental protection programs could be enhanced by using an integrated method, such as the one developed in this dissertation, to assess the cumulative impacts of chemical and physical stressors upon biological assemblages.

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HABITAT ASSESSMENT FIELD DATA SHEET

Station _____ RIVER BASIN _____

RIVER ~~NAME~~ _____ ECOREGION REFERENCE SITE _____

DATE _____ / _____ / _____ INVESTIGATOR _____

DESCRIBE SITE LOCATION _____

Comments:

Riffle/Run Prevalent Streams are those in moderate to high-gradient landscapes that sustain water velocities of approximately 30 cm/sec or greater. Natural streams in substrates primarily composed of coarse sediment particles (i.e., gravel or larger) or frequent coarse particulate aggregations along stream reaches.

Habitat Parameter	Category																				
	Optimal					Suboptimal					Marginal					Poor					
1. Instream Cover (Fish)	A mix of snags, submerged logs, undercut banks, rubble, or other stable habitat in greater than 50% of the sample area																				
SCORE	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
2. Epifaunal Substrate	<p>SNAGS + SUBMERGED LOGS low gradient streams. Const. rate ... of the reach</p> <p>20% 10-20% 5-10% 65%</p> <p>Well developed riffle run less well developed riffle lacking run</p>																				
SCORE	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
3. Embeddedness	<p>Snag - in reach 10' + 10' gravel, coarse rubble</p> <p>0-25% 5-30% 35-50% up to 50% 50-75% heavy 75+%</p>																				
SCORE	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
4. Channel Alteration	<p>Channelization or dredging absent or minimal; stream with normal pattern.</p> <p>Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.</p> <p>New embankments present on both banks; and 40 to 80% of stream reach channelized and disrupted.</p> <p>Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted.</p>																				
SCORE	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
5. Sediment Deposition	<p>Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.</p> <p>Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.</p> <p>Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.</p> <p>Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.</p>																				
SCORE	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0

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Habitat Parameter	Category																				
	Optimal					Suboptimal					Marginal					Poor					
6. Frequency of Riffles (or bends) / Velocity-Depth Combinations	Optimal: frequent riffles or bends in stream; velocity/depth patterns present. All 4 velocity/depth patterns present.					Suboptimal: infrequent riffles or bends; only 3 of 4 velocity/depth patterns present (i.e., slow (<0.3 m/s)-deep (>0.5 m); slow-shallow; fast-deep; fast-shallow).					Marginal: only 2 velocity/depth patterns present; usually lacking deep areas.					Generally all flat water or shallow riffles; poor habitat; dominated by one velocity/depth pattern.					
SCORE _____	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
7. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.					Water fills >75% of the available channel; or <25% of channel substrate is exposed.					Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.					Very little water in channel and mostly present as standing pools.					
SCORE _____	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
8. Bank Vegetative Protection (score each bank) Note: determine left or right side by facing downstream.	More than 90% of the streambank surfaces covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.					70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.					50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.					Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.					
SCORE ____ (LB)	Left Bank 10 9					8 7 6					5 4 3					2 1 0					
SCORE ____ (RB)	Right Bank 10 9					8 7 6					5 4 3					2 1 0					
9. Bank Stability (score each bank)	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.					Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.					Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.					Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.					
SCORE ____ (LB)	Left Bank 10 9					8 7 6					5 4 3					2 1 0					
SCORE ____ (RB)	Right Bank 10 9					8 7 6					5 4 3					2 1 0					
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.					Width of riparian zone 12-18 meters; human activities have impacted zone only minimally					Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.					Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.					
SCORE ____ (LB)	Left Bank 10 9					8 7 6					5 4 3					2 1 0					
SCORE ____ (RB)	Right Bank 10 9					8 7 6					5 4 3					2 1 0					

12/13/95

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Velocity

Massachusetts DEP | Physical Characterization/Water Quality Field Data Sheet

STATION: _____ STREAM NAME: _____ RIVER MILE: _____ DATE: 1/1

RIVER BASIN: _____ STREAM CLASSIFICATION: _____ INVESTIGATORS: _____

RECONN | HABITAT | INVERTEBRATE | FISH | FLOW | WQ

DESCRIBE LOCATION: _____

STREAM CHARACTERIZATION

- ☒ Subsystem Classification
- ☐ Tidal
- ☐ Lower Perennial
- ☐ Upper Perennial
- ☐ Intermittent
- ☐ Stream Type
- ☐ Coldwater
- ☐ Warmwater

Predominant Surrounding Land Use

Rt. Bank: 17m

Left Bank: 100m

15m

100m

RIPARIAN ZONE/INSTREAM FEATURES

☒ Predominant Surrounding Land Use

- ☐ Forest
- ☐ Field/Pasture
- ☐ Agricultural
- ☐ Residential
- ☐ Commercial
- ☐ Industrial
- ☐ Other

☒ Channelized Y N

☒ Dam Present Y N

☒ Local Water Erosion

- ☐ None
- ☐ Moderate
- ☐ Heavy

☒ Local Watershed NPS Pollution

- ☐ No evidence
- ☐ Some potential sources
- ☐ Obvious sources

☒ Estimated Stream Width

☒ Estimated Stream Depth (cm) 4

- ☐ Riffle
- ☐ Run
- ☐ Pool

☒ Estimated Fish Reach Length 7 m

☒ Canopy Cover

- ☐ Partly open
- ☐ Partly shaded
- ☐ Shaded

SEDIMENT/SUBSTRATE

- ☒ Odors
- ☐ Normal
- ☐ Sewage
- ☐ Petroleum
- ☐ Chemical
- ☐ Anaerobic
- ☐ None
- ☐ Other

- ☒ Oils
- ☐ Absent
- ☐ Slight
- ☐ Moderate
- ☐ Profuse
- ☐ Relict shells
- ☐ Other

☒ Deposits

- ☐ Sludge
- ☐ Sawdust
- ☐ Paper fiber
- ☐ Sand

☒ Are the underside of stones not deeply embedded black? Y N

INORGANIC SUBSTRATE COMPONENTS			ORGANIC SUBSTRATE COMPONENTS		
Substrate Type	Diameter	Percent Composition in Sampling Area	Substrate Type	Characteristic	Percent Composition in Sampling Area
Bedrock			Detritus	sticks, wood, coarse plant materials (CPOM)	
Boulder	>256mm (10 in)				
Cobble	64-256mm (2.5-10 in)				
Gravel	2-64mm (0.1-2.5 in)		Muck-mud	black, very fine organic (FPOM)	
Sand	0.06-2mm (gritty)				
Silt	0.004-0.06mm		Marl	grey, shell fragments	
Clay	<0.004mm (slick)				

WATER QUALITY

- ☒ Temperature 3 °C
- ☒ Dissolved Oxygen mg/L
- ☒ pH 7.5

- ☒ Water Odors
- ☐ Normal/None
- ☐ Sewage
- ☐ Petroleum
- ☐ Chemical
- ☐ Fish
- ☐ Other

☒ Water Surface Oils

- ☐ Slick
- ☐ Sheen
- ☐ Globbs
- ☐ Flecks
- ☐ None

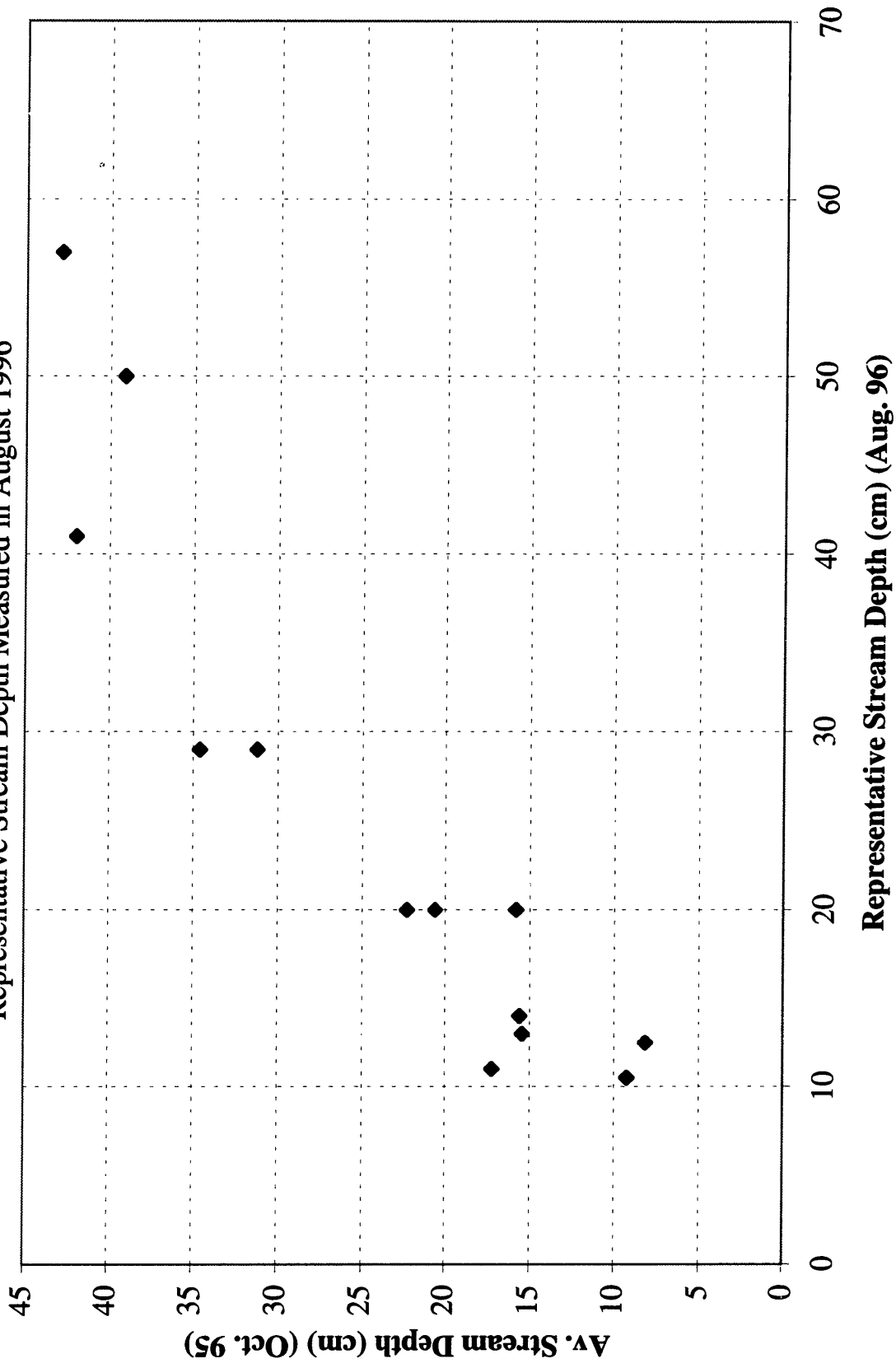
☒ Turbidity (if not measured)

- ☐ Clear
- ☐ Slightly turbid
- ☐ Turbid
- ☐ Opaque
- ☐ Water color

Conductivity at setting

W996069801\METH004 WPD

Appendix B. Comparison of Stream Depth Measurements
Average Stream Depth Measured in October 1995 v.
Representative Stream Depth Measured in August 1996



Appendix C. Long and Morgan (1990) vs. MacDonald (1992) ERL and ERM Values

Table C1. ERL and ERM Values for As, Cr, Cu, Pb and Zn (MacDonald 1992)

	As (% of 1990 values)	Cr	Cu	Pb	Zn
ERM (ppm)	70 (82%)	370 (255%)	270 (69%)	223 (203%)	410 (140%)
ERL (ppm)	8.2	81	34	46.7	150

Table C2. ERL and ERM Values for As, Cr, Cu, Pb and Zn (Long and Morgan 1990)

	As	Cr	Cu	Pb	Zn
ERM (ppm)	85	145	390	110	270
ERL (ppm)	33	80	70	35	120

“MacDonald (1992) generally doubled or tripled the amount of data in the ascending tables compiled by Long and Morgan (1990) mainly with new data from field studies and laboratory spiked-sediment bioassays. Also, MacDonald (1992) considered only estuarine and marine data, thereby deleting the freshwater data included in Long and Morgan (1990).” (Long and MacDonald 1992, p. 14-14). The thesis used MacDonald (1992)’s ERM values. The ERM values derived by MacDonald are generally higher (Cr, Pb, Zn 140% to 255% of 1990 values) although the ERM values for As and Cu are somewhat lower (69% to 82% of 1990 values).

Appendix D. The Complete Integrated Method for Assessing the Ecological Integrity of Urban Watersheds

Outline

1. Site Selection

Selection of Aberjona River watershed sites

Selection of “minimally-impaired” sites

2. Physical Habitat Assessment

3. Chemical Contamination Assessment

Sample Collection: Sediments and Epifaunal Habitats

Metal Analysis: Sediments and Epifaunal Habitats

Toxic Unit Analysis

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Site Selection

Selection of Aberjona River Watershed Sites

Seventeen locations in the watershed were chosen for study (Fig. 1.a): 6 are located along the Aberjona River from its source to its end in the Mystic Lakes; 8 sites are located throughout the Horn Pond subwatershed that carries water from the western part of the Aberjona watershed to the Aberjona River between Aberjona River sites Ab 5 and Ab 6; and 3 sites are located on the three largest remaining tributaries to the river. One site (H1) that is located in the Horn Pond subwatershed was not sampled for macroinvertebrates because it was dry in August 1996. Locations were chosen from a combination of U.S.G.S. maps (Reading, MA, 1987 and Boston, MA-North, 1985), street maps, and site visits. All sites are in walking distance (usually upstream) of road crossings. Four additional locations were chosen as “minimally-impaired” sites.

Selection of “Minimally-Impaired” Sites

The entire Aberjona watershed is influenced by heavy human-related development, and the watershed is part of the Boston Basin ecoregion (Griffith *et al.* 1994). One stream reach (site H7) within the watershed has suffered relatively little chemical or physical disturbance, and is located within a wooded parcel of conservation land (the Horn Pond recreational area). It was selected as a watershed reference site (Fig. 1a).

Additional reference sites were sought within the ecoregion (e.g., within least-disturbed areas of the Charles River Watershed and the Concord River Watershed). The criteria for the reference sites were: (1) relatively undeveloped headwaters, (2) no evidence of human alteration of the physical habitat, (3) wide (> 18m) vegetated riparian zones, and (4) no evidence of pollution (Hughes *et al.* 1990). Fifty candidate reference locations were selected from U.S.G.S. maps and from consultation with knowledgeable resource managers and scientists (personal communication with Scott Socolosky of MIT, Charles River Watershed Association, U.S. Army COE, U.S.G.S., the Massachusetts Department

of Environmental Protection, and U.S. EPA New England Regional Lab in Lexington, MA). These locations were selected because their geological and hydrological properties (stream order, drainage area and gradient) were similar to the sites in the Aberjona Watershed (U.S. Geological Survey maps: Reading, MA, 1987 and Boston, MA-North, 1985) and because minimal disturbance was suggested by available information. Four sites (named C, F, M and T) were determined to be of acceptable quality, and were chosen as “minimally-impaired” sites (Fig. 1.b).

Physical Habitat Assessment

Habitat Assessment

Physical habitat assessment was performed at 17 test sites and 4 reference sites (Fig. 1) according to the protocols used by the Massachusetts Department of Environmental Protection (Tetra Tech 1995) modified for slow-flow, low gradient streams. Ten habitat parameters (instream cover, epifaunal substrate, embeddedness, channel alteration, sediment deposition, variety of velocity-depth combinations, channel flow status, bank vegetative protection, bank stability, and riparian vegetative zone width) were scored from 0 to 20 (see Appendix A).

At the time of fish sampling (October 1995), measurements of stream depth and width were taken at 10m intervals for each stream reach sampled except for sites H1, Ab 3 and Ab 4. Physical habitat assessment performed the following August (1996) included one measurement of stream depth that was visually estimated to be representative of the stream reach’s depth. The average value of the stream depth measurements made in October 1995 correlate well (approximately 1:1 correlation) with representative measurements made in August 1996 (Appendix B).

Stream width, velocity and the following water characteristics were also measured: the concentration of dissolved oxygen (YSI Model 57 Oxygen Meter), the temperature (YSI Model 57 Oxygen Meter), the pH (Jenco Analog pH Meter 611), and conductivity (Cole

Parmer Conductivity Meter 1481-60). Characteristics of the substrate (% composition of various organic and inorganic components), and the percentage of sampling reach that is shaded (% shading) by overhanging vegetation were visually estimated.

Chemical Contamination Assessment

Sample Collection for Metal Analysis: Sediments and Epifaunal Habitats

After visual determination of the range of sediment grain size and organic content, areas of fine-grained, organic-rich sediments were collected at 17 test sites and 4 reference sites (Fig. 1) using a Russian corer. The torpedo-shaped, aluminum corer head was gently inserted into the top 2-3 cm of sediment and twisted 180° to capture the sample. The corer was pulled out of the sediment, twisted again to expose the sample, and sediment was scooped off the corer with a teflon spatula and stored in glass jars.

Epifaunal habitats (submerged vegetation, vegetation-covered rocks, overhanging bank vegetation, and undercut bank roots) were sampled and stored in glass jars. Watercress was sampled at sites Ab 1, H4, H5 and Tr 2. Submerged vegetation was sampled at sites Ab 3, H7 and T. Undercut bank roots were sampled at sites Ab 2, Ab 5, Ab 6, H3, H8 and Tr 3. Vegetation was scraped from rocks at sites H2, H3, H6, C and F. Silty overhanging bank vegetation was sampled at site Ab 4. A mixture of watercress and submerged vegetation was sampled at site Tr 1 and a mixture of emergent and overhanging bank vegetation was sampled at site M.

Jars containing sediment and habitat samples were immediately placed on ice and kept refrigerated (12 hours to 2 days) until they were dried at 85°C to a constant weight (approximately 1-2 days).

Metal Analysis: Sediments and Epifaunal Habitats

Five (5.0) grams of each dried sample were pulverized in a Spex Mixer cartridge with a silicon carbide ball for 5 minutes. One-half gram (0.5 g) of copolywax (TM) binder

was added to the sample and mixed again for 1 minute. This mixture was poured into a 31mm diameter aluminum sample cup and pressed for 1 minute using 12 metric tons of force in an evacuable die. Elemental concentrations in pellets were analyzed by X-Ray Fluorescence (XRF) using a Philips PW1480 wavelength dispersive XRF and Uniquant data processing software.

Toxic Unit Analysis

Concentrations of As, Cr, Cu, Pb and Zn were normalized by sediment quality benchmarks (Table D.1, Expected Risk Low (ERL) and Expected Risk-Median (ERM) values, Long and Morgan 1990 updated by MacDonald 1992). The resulting risk ratios (environmental concentration/ benchmark concentrations) are dimensionless, and are referred to as toxic units. Ratios in excess of 1 occur when concentrations in sediments are higher than benchmarks.

Table D.1. ERL and ERM Values for As, Cr, Cu, Pb and Zn (MacDonald 1992)

	As	Cr	Cu	Pb	Zn
ERM (ppm)	70	370	270	223	410
ERL (ppm)	8.2	81	34	46.7	150

Analysis of Metal Concentrations in Fish

Fish were collected by wading method electroshocking (see below: Fish Sampling). Fish retained for metal analysis were stored frozen in polyethylene bags until March 1996 when they were thawed, processed, and analyzed for metals by instrumental neutron activation. In the process of freezing and thawing, a small amount of liquid was released from the fish and stuck to the polyethylene bags. As a result, the masses of the fish decreased slightly, usually less than 8-15%. Fish lengths were measured from the tip of the mouth to the tip of the tail (full length) and from the tip of the mouth to the fork of the

tail (fork length). In cases where fish tails were torn and the fork could not be distinguished, only the full length was recorded. Using an Omni Mixer Homogenizer with a titanium cutting blade, fish were ground into a pulp inside acid-washed polyethylene bottles (with the caps cut off). Less than 0.2 g of sample was transferred into acid-washed sample bags with nickel forceps and put into vials for neutron activation analysis. Approximately one gram of the homogenous fish mixture was transferred into pre-weighed aluminum tins and weighed before and after drying. (Dry weights were recorded until weight was constant.) Samples were dried in a 100°C oven at least overnight.

The samples were irradiated inside the Massachusetts Institute of Technology (MIT) nuclear reactor with a thermal neutron flux of $8 \times 10^{12} \text{ n cm}^{-2} \text{ sec}^{-1}$ for 12 hours. The samples were then cooled for 2-3 days and transferred to clean polyethylene bags for counting. Gamma ray emissions were measured with high purity germanium detectors coupled to 8192-channel pulse-height analyzers (Canberra, CT). To detect the g-peaks of each isotope, the spectra were analyzed using the computer program ND 9900 Genie run on a VMS 200 computer (Canberra, CT). A fly ash standard reference material (SRM 1633), purchased from the National Institute Standards and Technology (NIST), was used to calculate elemental concentrations. Two other reference materials, SRM 1571 (orchard leaves) and SRM 1577a (bovine liver), were used to check the system stability. Bovine liver was put into polyethylene bottles, ground, and put in contact with the titanium blade and analyzed for quality control. All samples, standards and control samples were counted for 12 hours at a constant geometry. Additional details of the analytical procedure have been published elsewhere (Olmez 1989).

Concentrations of As, Cr and Zn in fish were also measured by the U.S. EPA (data supplied by Mary Garren, U.S. EPA Region 1, data collected as part of ongoing studies of the Wells G and H Superfund Site). Data from the EPA study is included in Figure 3.8.

Biological Sampling

Fish Sampling

Seventeen locations were selected in the Aberjona Watershed (Fig. 1.a) and fish populations were sampled (September 30-October 9, 1995) at each site by wading method electroshocking using a Coffelt CPS-system DC pulsed electroshocker. To estimate population densities, a removal method was performed using two runs (White et al. 1992). In cases where the catch was very low (only a few individuals), one single run was carried out. After anaesthetizing the fish (MS 222), each was identified to the species level, its fork length was measured and obvious signs of gross disease were recorded. Some fish were taken back to the laboratory for metal analysis. The remainder were allowed to recover in stream water and were put back into the stream at the location where they were caught.

Electroshocking was successful at 16 locations. At one location (Ab 6), the water was too deep and turbid for effective collection. Of the 16 successfully fished locations, 5 sites are located on the Aberjona River, 3 sites are located on small tributaries to the Aberjona River (Halls Brook, North Woburn Creek, and Sweetwater Brook), and 8 are located in the Horn Pond subwatershed. Fish were caught at 10 locations and no fish were observed at 6 locations.

Macroinvertebrate Sampling

Macroinvertebrate sampling was performed at 16 locations (H1 was not included because it was dry at the time of sampling) in the Aberjona watershed and at 4 additional “reference” locations in August 1996 according to the protocols used by the Massachusetts Department of Environmental Protection (Tetra Tech 1995). The protocols are similar to Method 7.2 “Multihabitat Approach; D-Frame Dip Net” described by Barbour *et al.* (1997). Briefly, the multihabitat approach consists of sampling macroinvertebrate habitats, such as cobble, snags, vegetated banks, submerged macrophytes and sand, in proportion to their

visually-estimated representation in the stream. The stream is visually assessed and rough percentages are assigned to the proportion of available instream habitat belonging to each category. Samples are collected by kicking the substrate or jabbing with a rectangular dip net (0.5 m width, 0.3 m height). A total of 10 jabs (or kicks) were taken from all major productive habitat types in the reach resulting in sampling of approximately 2.5 m² of habitat. In this study, duplicate samples were collected at 3 randomly chosen locations.

Macroinvertebrate Subsampling and Species Identification

Samples were sorted and subsampled in the laboratory according to the protocols used by the Massachusetts Department of Environmental Protection (Tetra Tech 1995). Whole samples (all material collected in the 10 jabs or kicks, including benthic macroinvertebrates, bits of vegetation, small pebbles and sand) were rinsed; large organic material was rinsed, visually inspected, and discarded. Samples were spread evenly across trays marked with grids, and subsampled randomly. Organisms were sorted under a dissecting microscope. Subsamples of 100 plus/minus 20 organisms preserved in 95% alcohol were identified to the lowest practical taxon, generally genus or species. A representative of every species was maintained in a reference collection.

Metrics Used To Characterize Biological Condition

Fish Assemblages

The biological condition of the fish assemblages of the Aberjona watershed was characterized using the 12 metrics adapted by Miller *et al.* (1986) from Karr's (1981) index of biotic integrity (IBI) for use in the Merrimac and Connecticut River Drainages in Massachusetts and New Hampshire. The metrics were: 1) Total number of fish species, 2) Number and identity of native water column species, 3) Number and identity of native benthic insectivorous species (excludes insectivorous sucker species), 4) Number and identity of native sucker species, 5) Number and identity of native intolerant species, 6)

Proportion of individuals as white sucker, 7) Proportion of individuals as omnivores (excludes white sucker), 8) Proportion of individuals as insectivores, 9) Proportion of individuals as top carnivores, 10) Density of individuals in sample, 11) Proportion of individuals as hybrids, and 12) Proportion of individuals with disease, tumors, fin damage or other anomalies. The metrics were calculated and their values at sites with varying degrees of physical and chemical disturbance were compared to the values calculated for each metric at the less-disturbed site H7.

Macroinvertebrate Assemblages

Biological condition was characterized with benthic metrics (Table D.2) selected from among four categories: taxa richness, composition metrics, tolerance/intolerance measures, and feeding measures.

Table D.2. Set of candidate benthic metrics

<u>Category</u>	<u>Metric</u>	<u>Expected Response to Perturbation</u>	<u>Definition</u>
Richness	Total No. taxa	Decrease	Measures the overall variety of the macroinvertebrate assemblage
	No. EPT taxa	Decrease	Number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)
Composition	% EPT	Decrease	Percent of the composite of mayfly, stonefly, and caddisfly larvae (taxa sensitive to pollution and other stressors)
	% Orthocladiinae to chironomids	Increase	Percent of chironomids in the subfamily Orthocladiinae
Tolerance	% Dominant taxon	Increase	Measures the dominance of the single most abundant taxon.
	Hilsenhoff Biotic Index	Increase	Uses tolerance values to weight abundance in an estimate of overall pollution. Originally designed to evaluate organic pollution.
	% Hydropsychidae Increase to Trichoptera		Relative abundance of pollution tolerant caddisflies (metric could also be regarded as a composition measure)
	No. of Intolerant Taxa	Decrease	Number of taxa with tolerance scores ≤ 3 .
	% Tolerant Organisms	Increase	Percent of sample composed of organisms with tolerance scores ≥ 7 .
Feeding/Habit	% Filterers	Variable	Percent of the macrobenthos that filter FPOM from either the water column or the sediment
	No. Scraper & Piercer taxa	Decrease	Number of taxa feeding upon living plant material either by scraping periphyton or by piercing macrophytes.
	% Clingers	Decrease	Percent of sample composed of organisms classified predominantly as clingers (Merritt & Cummins 1984).

Characterization of the Relationship of Biological Condition to Habitat Condition and Habitat Condition

Fish Assemblages

Application of the Barbour *et al.* (1997) graphical framework to the Aberjona watershed required the selection of appropriate measures of habitat condition and biological condition. The number of native fish species caught at a site was chosen as a measure of biological condition. An index of habitat condition was constructed that was: (a) normalized to range from 0 to 1, (b) equal to 0 if stream depth was below the threshold value of 20 cm, and (c) that was evenly weighted between stream depth and instream cover score for stream depth values above 20 cm:

HABITAT CONDITION INDEX

$$\begin{aligned}\text{Habitat Condition} &= 0 \text{ if stream depth} < 20. \\ &= 0.5 [(SD / \text{max SD}) + (IC / \text{max IC})]\end{aligned}$$

where

SD = stream depth

max SD = max stream depth of all sites (= 62 cm)

IC = instream cover score

max IC = maximum instream cover score of all sites (= 18)

The relationship of biological condition to overall habitat quality, the availability of instream cover, and stream depth was evaluated graphically. Chemical contamination was quantified using the total toxic units score approach (Long and Morgan 1990, MacDonald 1992, Long and MacDonald 1992). The relationship of biological condition to habitat condition and chemical contamination was evaluated using the Barbour *et al.* (1997) graphical framework: the number of native fish species caught at a site was plotted versus the habitat condition index. The following predictions were tested: (a) that in the absence of chemical contamination (reflected by low total toxic unit scores), biological condition would vary from good to poor as habitat condition varied from good to poor, and that (b) biological degradation beyond that predicted by habitat condition could be explained by contamination (reflected by high total toxic units scores).

Macroinvertebrate Assemblages

The relationship of each benthic metric and an aggregate macroinvertebrate index (described below) to habitat degradation and/or sediment contamination was evaluated by grouping sites by impairment categories (Table D.3), and comparing the values of benthic metrics and the aggregate index across categories using box & whisker plots. This method allows comparison of the median, 25th and 75th percentile values, and the range between categories. In addition, *t-tests* of the significance of the difference between categories and linear regression of benthic metric values and the aggregate index upon physical and chemical characteristics were also performed.

<u>Table D.3. Criteria for Site Categorization</u>			
<u>Category</u>	<u>Total Habitat Score</u>	<u>Total Toxic Units for Habitat Sample</u>	<u>Comment</u>
Ref (Reference)	155-185	0.5 - 1	outside watershed
SomeHab (Some Habitat Impairment; Horn Pond subwatershed sites)	97-120, (169)	0.5 - 3.5	Site H7 has a habitat score in the reference site range (169). It has a slightly higher Toxic Unit score (2). It was grouped geographically with the Horn Pond subwatershed sites.
Hab (Greatest Habitat Impairment)	60-81	1 - 2.5	
HabCon (Habitat Impairment and Contamination)	80-100	7 - 13	
Con (Greatest Contamination)	130-155	17 - 23	

Construction of an aggregate macroinvertebrate index

An aggregate index of the biological condition of macroinvertebrate assemblages was developed from the candidate benthic metrics (methods developed from Barbour *et al.* 1996b, Gibson *et al.* 1996). The requirements for inclusion of metrics in an aggregate index were the demonstrated ability to discriminate impaired from unimpaired conditions, non-redundancy with other metrics, and the inclusion of metrics from all four categories (taxa richness, composition, tolerance, feeding measures). One aspect of discriminatory ability is numerical: Barbour *et al.* (1996b) included in an aggregate index only those metrics with a median (for reference sites) of 6 taxa or more for taxa richness metrics and a median (for reference sites) of 15% or more for percentage metrics expected to decrease with impairment. These values were reasonable for the Boston Basin ecoregion. Using the four reference sites located outside of the Aberjona watershed to calculate median reference values, we additionally required that percentage metrics that increase with impairment have a median (for reference sites) of less than 85%. (One metric was included that had a median of 5.5 for the reference sites.) The second aspect of discriminatory ability - sensitivity to impairment - was evaluated as described below: differences between metric values for reference sites and impaired sites were evaluated by comparing the median, the 25th and 75th percentile values, and the range between categories. Metric redundancy was evaluated using a Pearson Correlation matrix, and the results of bivariate scatterplots for metrics with correlations $r > 0.7$ (Barbour *et al.* 1996b). Metrics that met the criteria for inclusion in the aggregate macroinvertebrate index are referred to as core metrics.

Core metrics were normalized into unitless scores. The scoring concept is a modification of the methods of the Index of Biotic Integrity (e.g. Karr *et al.* 1986, Karr 1991). The 95th percentile value of metrics that decrease in response to perturbation was calculated. The region from 0 to the 95th percentile value was quadrisected and each region was scored 0, 2, 4, or 6, with high quality sites receiving the highest scores. The 5th

percentile of metrics that increase in response to perturbation (e.g., % Hydropsychidae to Trichoptera, % Oligochaeta) was calculated. The region from the 5th percentile value to the maximum value observed across sites was quadrisected and each region was scored 0, 2, 4, or 6, with low values (indicative of high quality sites) receiving high scores. The aggregate index was the sum of the scores for each core metric.

Appendix E. A Poem by Harold F. Hemond

A lady came from Jefferson's School,
At the Parsons Lab to study and tool.

For research she expressed her wishes,
To study Aberjona fishes.

She decided she would test their metal,
By cooking in the nuclear kettle
And after waiting a few days,
To measure all their gamma rays.

Invertebrates too, she gathered all,
And put them into alcohol.
Nymph and larva, small and wet,
Dozens ended in her net.

Sediments, and substrate too,
Were things of which Catriona knew.
Pressed in pellets, or digested,
Of their toxins knowledge wrested.

Now at last the story's clear,
And of your accomplishments we cheer.
To the expert on the fishes.
Congratulations and best wishes.

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Hair Analysis Does Not Support Hypothesized Arsenic and Chromium Exposure from Drinking Water in Woburn, Massachusetts

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We hypothesized that residents of Woburn, Massachusetts, had been exposed to as much as 70 µg/l of arsenic (As) and 240 µg/l of chromium (Cr) in drinking water from municipal supply wells G and H. To test this hypothesis, we measured the concentrations of As and Cr in 82 hair samples donated by 56 Woburn residents. Thirty-six samples were cut between 1964 and 1979, the period during which wells G and H were in operation. The remainder were cut either before 1964 (1938–1963; $n = 26$) or after 1979 (1982–1994; $n = 20$). Washed hair samples were analyzed by instrumental neutron activation. Exposure to the well water—measured as access—was estimated using well pumping records and a model of the Woburn water distribution system. Our results show that access to wells G and H water was not significantly correlated (95% confidence interval) with As and Cr concentrations measured in the hair of Woburn residents, but As concentrations have declined significantly over the last half century. Linear regression of As concentrations (micrograms per gram) upon year of haircut and access to wells G and H water yielded a standard coefficient for year of -0.0074 ± 0.0017 (standard error; $p = 2.5 \times 10^{-5}$) and -0.12 ± 0.10 ($p = 0.22$) for access. The r^2 value for the model was 0.19. The geometric mean concentrations (geometric standard deviation) of As and Cr in the hair of residents who had access (i.e., relative access estimate >0) to wells G and H water ($n = 27$) were 0.14 (2.6) and 2.29 (1.8) µg/g, respectively; the geometric mean concentrations of As and Cr in all of the hair samples from residents who did not have access (1938–1994; $n = 55$) were 0.13 (3.0) and 2.19 (2.0) µg/g, respectively. **Key words:** arsenic, chromium, groundwater, human hair, Woburn, Massachusetts.

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Based on our knowledge of As and Cr distribution and transport in the watershed, we considered the possibility that water from municipal supply wells G and H may have been contaminated with these elements (13). Wells G and H were used between 1964 and 1979 to supplement supplies to east Woburn, providing as much as 35% of the city's water (14). Although little information is available on the elemental composition of wells G and H water, the hypothesis that the well water contained elevated concentrations of As and Cr—and possibly other elements—was supported by two discoveries: as much as 60% of the recharge water for the wells came from the Aberjona river (15), and during the 15 years that the wells were in use, the peak concentrations of As and Cr in the river were estimated, on the basis of lake sediment analysis, to be on the order of 120 and 400 µg/l, respectively (12). This suggested that residents who consumed water from wells G and H could have been exposed to as much as 70 µg/l As and 240 µg/l Cr (the drinking water quality standard for As and Cr are currently 50 µg/l and 100 µg/l, respectively).

If Woburn residents ingested these elevated concentrations of As and Cr, a record of this exposure would be stored in hair that was grown during the period of exposure. Once in the body, As and Cr accumulate in hair, binding strongly to keratin in hair strands (16–18). Retrospective studies performed to determine whether people who had been dead for a long time had experienced acute As exposures [e.g., Napoleon, and the mayor

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Chemical manufacturing and leather tanning activities on the Aberjona River watershed in eastern Massachusetts (Fig. 1) have left a legacy of environmental contamination. The earliest records of contamination date to the 1870s when it was reported that tanneries in Woburn were discharging their wastes into tributaries of Horn Pond, which then served as a drinking water supply for Woburn (1–3). Since that time, reports of additional contamination resulting from leather tanning, chemical manufacturing, and other industrial activities on the watershed have been made with alarming regularity (4,5). As a result, there are over 100 sites in the watershed—including two EPA Superfund sites (Industri-Plex and wells G and H)—that are now being investigated for the presence of hazardous man-made chemicals (6).

Although many of the chemicals identified at these disposal sites (e.g., gasoline, organic solvents, pesticides, plasticizers) are from contemporary sources, large quantities of toxic elements released by historical tanning and chemical manufacturing operations have been discovered. For example, at four sites in Woburn and one in Winchester, tanning wastes containing elevated concentrations of chromium, arsenic, lead, and other elements have been detected (5). In addition, at the Industri-Plex site, it is estimated that

several tons of arsenic, chromium, and lead were released during the manufacture of arsenic-based pesticides, sulfuric acid, and glues (5,7).

The concern that arsenic and chromium residues may be moving off these sites by leaching into groundwater and surface waters has led to several recent investigations. Davis et al. (8) reported that the sediments of Halls Brook Pond, which is immediately downgradient of the Industri-Plex site, contain as much as 3,000 mg/kg As and 1,400 mg/kg Cr [average crustal abundances are on the order of 1–10 mg/kg for As and 10–100 mg/kg for Cr (9,10)]. Knox (11) found that many sediments along the Aberjona river, and in particular a riparian wetland area within the wells G and H site boundary (see Fig. 1), contained elevated concentrations of these elements. Spliethoff and Hemond (12) reported that the sediments of Upper Mystic Lake, into which the Aberjona drains, contained the depositional record of As and Cr dating back to the mid-1800s. Peak concentrations in these sediments were found to be as high as ~1,800 mg/kg As and ~6,000 mg/kg Cr.

To date, no work has been done to determine whether residents of this watershed (population ~75,000) have been exposed to elevated levels of As and Cr.

of Zurich who died in 1360 (19) suggest that As in hair may be used as a biomarker, even in samples that are several hundred years old. Although similar studies of the Cr content of historic hair samples have not been reported, it has been demonstrated that Cr within hair strands is not easily removed, even upon washing with shampoos and organic solvents (20); thus, it is reasonable to expect that Cr levels in decades-old hair samples will reflect the levels at the time Cr was initially incorporated in the hair. The relationship between As and Cr in hair and drinking water has been demonstrated in studies in which populations exposed to elevated levels of As and Cr in their drinking water were found to have significantly higher concentrations of these elements in their hair than control populations (21–24).

In this study we compared As and Cr concentrations measured in the hair of Woburn residents with estimates of each donor's access to water from wells G and H. We posited that if As and Cr were present in the well water, then individuals consuming the water would have incorporated As and Cr into their hair in proportion to the amount of well water consumed. We used instrumental neutron activation analysis to determine the As and Cr concentrations of the hair samples, a model of the Woburn water distribution system to estimate access to wells G and H, and linear regression analysis to determine whether As and Cr concentrations in hair samples were dependent upon access to well water in a statistically significant manner.

Methods

Hair collection. The samples used in this study were obtained from Woburn residents who saved locks of hair cut from their children or themselves before, during, and/or after the period when wells G and H were in use. The samples, many of which had been stored in photograph albums and scrapbooks, were placed individually in air-tight polyethylene bags and transported to the laboratory for analysis. For each sample, the donor's sex, age, and address at the time the hair was cut were recorded, as were the month and year of the haircut. For 14 of the samples collected between 1964 and 1979, the month that the hair was cut was not known so only the year was recorded. A total of 82 samples were collected, representing 56 donors and 34 different residences. Twenty-six of the samples were cut between 1938 and 1963, 36 were cut between 1964 and 1979 (the period during which wells G and H were in operation), and 20 were cut between 1982 and 1994. Seventy-two samples were from children between the ages of 1 month and

19 years; the remaining samples were from adults over 19 years of age.

Elemental analysis. To distinguish elements incorporated in the hair during growth from those that were deposited on the outside of hair strands, the samples initially were washed with both acetone and

water according to a hair cleaning procedure described elsewhere (25). After drying in a fume hood at room temperature, the samples were irradiated with a thermal neutron flux of 8×10^{12} n/cm²/sec for 12 hr. The samples were then cooled for 2–3 days, washed again with acetone, and transferred

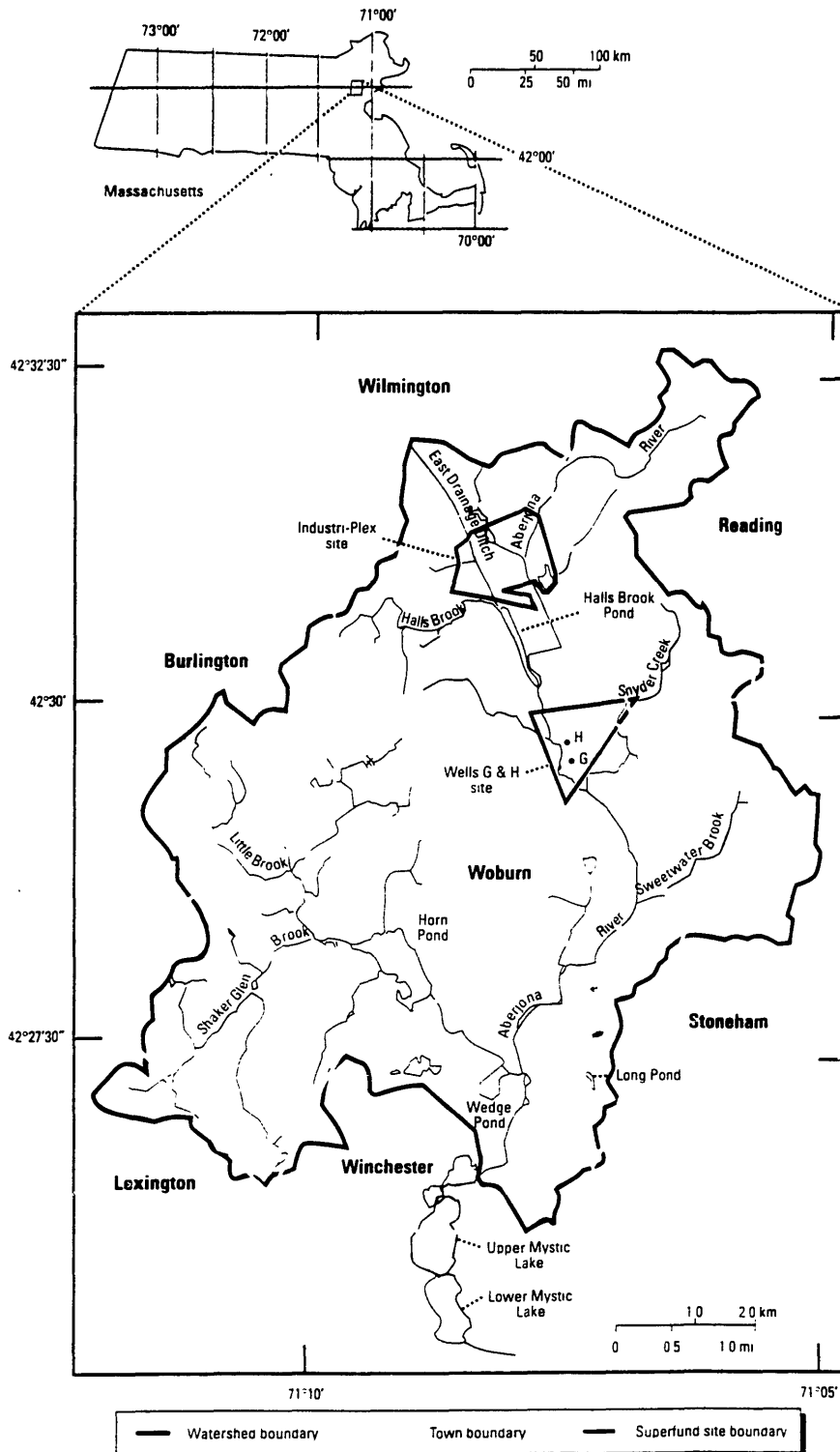


Figure 1. Map of the Aberjona watershed.

to clean polyethylene bags for counting. All of the gamma-ray spectroscopy was performed using four high purity germanium detectors coupled to an 8192-channel Genie system operating on a VAX 3100 workstation. Elemental concentrations were determined using custom-made interactive peak fitting and analysis software (all hardware and software were from Canberra Industries, Inc, Meriden, CT). A fly ash standard reference material (SRM 1633), purchased from the National Institute of Standards and Technology (Gaithersburg, MD), was used to calculate elemental concentrations. Two other reference materials, SRM 1571 (orchard leaves) and SRM 1577a (bovine liver), were used to check the system stability. All samples, standards, and control samples were counted for 12 hr at a constant geometry. Additional details of the analytical procedure have been published elsewhere (26).

Exposure estimation. An accurate method for relating As and Cr concentrations measured in hair samples to exposure to these elements in water from wells G and H would have been to determine the amount of each element imbibed during the period when each donor's hair sample was grown. However, because As and Cr were not measured in water from wells G and H when the wells were in use and because the volume of water from wells G and H consumed by each hair donor were also unknown, measurements of actual exposure to As and Cr could not be made. Instead, we investigated the relationship between As and Cr concentrations in hair samples and the donors' relative access to water from wells G and H. Access is defined here as the ratio of the amount of water from wells G and H to the total amount of municipal water delivered to a hair donor's house during the period when the hair sample was grown. Access was calculated by combining estimates of the period of time when each hair sample was grown and the fraction of water supplied to a residence by wells G and H. The methods used in determining these estimates are described below.

The hair growth period was estimated by dividing the length of the hair by an assumed growth rate of 1 cm/month (27). The dates corresponding to the oldest and youngest parts of the hair strands (i.e., the dates between which As and Cr exposure would have been recorded in the hair sample) were calculated using the date that the hair was cut and estimates of the length of hair on a donor's head remaining after cutting. If the length of hair remaining after cutting was unknown (i.e., it was not supplied by the donor or the donor's parents), lengths were assumed based on the sex and age of the

donor. For boys, the length of hair remaining after cutting was assumed to be 4–6 cm; for girls younger and older than 2 years of age, values of 4–6 cm and 8–12 cm, respectively, were used.

For the 14 samples for which only the year of collection was known, the growth period was deliberately overestimated to ensure that the true growth period for the hair was included within the estimated growth period. The beginning of the hair growth period was calculated using the earliest possible month of hair cutting (January) and the longest estimate of hair length; the end of the hair growth period was calculated using the latest possible month (December) and the shortest estimate of hair length. The disadvantage of this technique is that the true growth period for a sample was actually shorter than the estimated growth period; therefore, episodes of low or high access to wells G and H water cannot be resolved. Despite this distortion, this method was chosen over the alternative, which was to arbitrarily choose a shorter growth period that may or may not represent the true growth period.

Estimates of well water access were based on a model of the city's water distribution system developed by Murphy (14). In the original model, well pumping records and data on the water distribution system in Woburn were used to estimate the relative amounts of water arriving at a particular location from wells G and H and the Horn Pond well fields (Fig. 1). Recently, hydraulic mixing calculations were incorporated into the model, and source apportionment indices for various distribution nodes located throughout the city were calculated for each month that wells G and H were in use (28). In estimating the amount of water from wells G and H to which hair donors had access, we divided the sum of these published monthly exposure values by the number of months that the hair was estimated to have grown. Average monthly access estimates ranged from 0 (none of the water delivered to a residence during the period of hair growth was from wells G and H) to 1 (all of the water delivered to a residence during the period of hair growth was supplied by wells G and H).

Results

Arsenic. Overall, our results indicate that donors who had access to water from wells G and H did not have higher concentrations of As in their hair than donors who did not have access. A plot of As concentrations in hair versus a donor's relative access to wells G and H water is shown in Figure 2A. The figure shows that there is no apparent correlation between access and As concentrations

measured in hair. A least squares regression line through the data has a slope that is not significantly different from zero. Interestingly, a plot of the As concentrations in all hair samples versus the year that the hair was cut (Figure 3A) indicates that As levels in the hair of Woburn residents have decreased over the last 50 years. To determine whether there was a relationship between As concentrations in hair and a donor's relative access once this temporal variation in As concentrations was accounted for, multivariable regression was performed. Since As concentrations are linearly related to year (Figure 3A), the form of the regression was

$$[\text{As}]_{\text{hair}} = B_0 + B_1 \times \text{year} + B_2 \times \text{access} + \text{error}$$

The results in Table 1 show that As concentrations in hair were statistically dependent (at the 95% confidence level) upon year, but not upon access. The R^2 value for the model was 0.19.

The arithmetic and geometric mean concentrations of As in hair samples are shown in Table 2. The samples are grouped according to the year that the hair was cut and the donor's access to wells G and H water. As is evident from Figure 4A, the As concentrations in the hair of donors with access (i.e., relative access estimate > 0) and donors without access to the well water are log-normally distributed; thus, the geometric mean concentrations are the more appropriate measure to compare the different groups. The arithmetic mean concentrations were calculated so that we could compare our results with other studies that have reported their findings in terms of arithmetic mean concentrations. The geometric mean concentration (GSD) of As in the hair of residents who had access to water from wells G and H (1964–1979, with access) was 0.14 (2.6) $\mu\text{g/g}$ ($n = 27$), while for the concurrent control group (1964–1979, no access) the mean concentration was 0.18 (1.6) $\mu\text{g/g}$ ($n = 9$) (Table 2). The mean concentration of As in the hair of all of the controls (1938–1994, no access) was 0.13 (3.0) $\mu\text{g/g}$ ($n = 55$).

Chromium. The plots of Cr concentrations in hair samples versus relative access to water from wells G and H and versus the year that the hair was cut are shown in Figures 2B and 3B, respectively. Although there appears to be an increase in the concentrations of Cr in hair just after well G was turned on, well water access estimates and concentrations of Cr in hair are not significantly correlated. A least squares regression line through the data has a slope that is not significantly different from zero. Thus, as was the case for As, there is no indication that increased access to water

from wells G and H resulted in higher concentrations of Cr in hair.

The mean concentrations of Cr in the hair samples are shown in Table 2. Figure 4B shows that the Cr data is not fitted well by

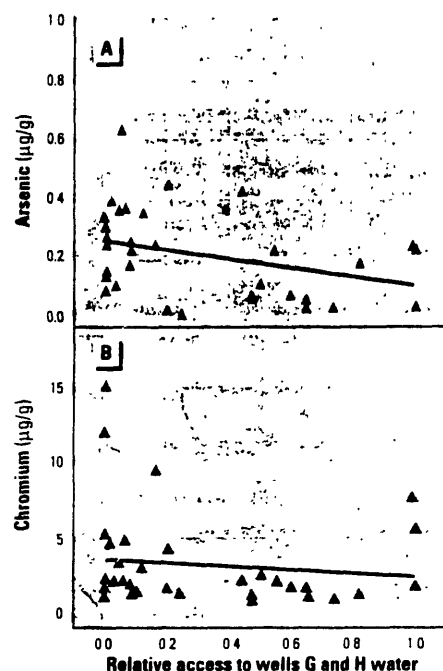


Figure 2. Concentrations of (A) As ($y = -0.15x + 0.25$, $r = 0.36$) and Cr ($y = -1.20x + 3.59$, $r = 0.12$) in hair samples from Woburn residents versus access to water from wells G and H (see Methods for explanation). The results shown are for hair samples that were cut between 1964 and 1979, the period during which wells G and H were in operation. A least squares regression line is drawn in each plot to show the trend in the data.

either a standard or a log-normal distribution; thus, the arithmetic and geometric mean concentrations are presented in Table 2. The geometric mean concentration (GSD) of Cr

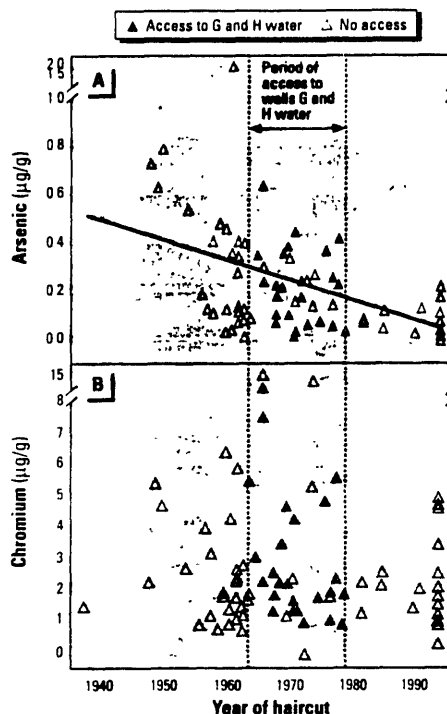


Figure 3. Concentrations of (A) As and (B) Cr in hair samples from Woburn residents versus the year of the haircut. Hair donors that had and did not have access to water from municipal supply wells G and H are represented by the closed and open symbols, respectively. Well G was in operation between 1964 and 1979; well H was in operation between 1967 and 1979. Line in (A) is least squares regression ($y = -0.0083x + 16.52$, $r = 0.45$).

in the hair of residents who had access to wells G and H water (1964–1979, with access) was $2.29 (1.8) \mu\text{g/g}$ ($n = 27$), while for the concurrent control group (1964–1979, no access) the mean concentration was $3.11 (2.4) \mu\text{g/g}$ ($n = 9$). The mean concentration of Cr in the hair of all of the controls (1938–1994, no access) was $2.19 (2.0) \mu\text{g/g}$ ($n = 55$). The arithmetic mean concentrations for these three groups were $2.80 \pm 2.08 \mu\text{g/g}$ ($n = 27$), $4.78 \pm 5.14 \mu\text{g/g}$ ($n = 9$), and $2.82 \pm 2.59 \mu\text{g/g}$ ($n = 55$), respectively.

Discussion

The concentrations of As and Cr in hair have been reported in many different studies. As shown in Table 3, As and Cr in hair have both been reported at concentrations ranging from $<0.1 \mu\text{g/g}$ to $>30 \mu\text{g/g}$ (29). A review of studies in which the hair of only healthy individuals was analyzed suggests that the normal ranges for As and Cr are roughly the same: 0.10 – $3.7 \mu\text{g/g}$ (17–19,29–34). In comparing our results with those in Table 3, we find that the concentrations of As and Cr in the hair of Woburn residents are consistent with what others have reported. The arithmetic mean concentrations of As and Cr in the hair of Woburn residents who did not

Table 1. Linear regression of As concentrations in hair upon year of haircut and access to wells G and H water

Parameter	Parameter Estimate	Standard error	p-value
Year of haircut	-0.0074	0.0017	2.5×10^{-5}
Access to wells G and H	-0.12	0.10	0.22

Table 2. Concentrations of As and Cr in hair samples from residents of Woburn, Massachusetts, with and without access to water from wells G and H

Group	Number	Arsenic ($\mu\text{g/g}$)				Chromium ($\mu\text{g/g}$)			
		A-mean \pm SD ^a	G-mean (GSD) ^b	Median	Range	A-mean \pm SD ^a	G-mean (GSD) ^b	Median	Range
1938–1994, no access	55	0.22 ± 0.30	0.13 (3.0)	0.13	0.009–1.9	2.82 ± 2.59	2.19 (2.0)	2.1	0.34–15
Male	19	0.34 ± 0.44	0.22 (2.6)	0.22	0.009–1.9	3.80 ± 3.81	2.56 (2.5)	2.3	0.34–15
Female	36	0.16 ± 0.16	0.10 (1.7)	0.10	0.018–0.62	2.35 ± 1.40	2.02 (3.0)	1.9	0.73–6.4
1964–1979, with access	27	0.21 ± 0.16	0.14 (2.6)	0.22	0.017–0.63	2.80 ± 2.08	2.29 (1.8)	2.0	0.93–9.5
Male	7	0.28 ± 0.17	0.24 (1.7)	0.23	0.11–0.63	3.00 ± 2.06	2.59 (1.7)	2.3	1.3–7.5
Female	20	0.19 ± 0.15	0.12 (2.8)	0.14	0.017–0.45	2.73 ± 2.14	2.19 (1.9)	1.8	0.93–9.5
1964–1979, no access	9	0.20 ± 0.09	0.18 (1.6)	0.16	0.091–0.34	4.78 ± 5.14	3.11 (2.4)	1.9	1.7–15
Male	6	0.21 ± 0.07	0.20 (1.4)	0.21	0.14–0.31	6.37 ± 5.76	4.37 (2.4)	3.8	1.7–15
Female	3	0.18 ± 0.14	0.14 (1.8)	0.10	0.091–0.34	1.60 ± 0.36	1.58 (1.2)	1.7	1.2–1.9
1964–1979, all samples	36	0.21 ± 0.14	0.15 (2.4)	0.20	0.017–0.63	3.19 ± 3.16	2.47 (2.0)	2.0	0.93–15
Male	13	0.25 ± 0.13	0.22 (1.5)	0.23	0.11–0.63	4.55 ± 4.36	3.30 (2.1)	2.4	1.3–15
Female	23	0.18 ± 0.15	0.12 (2.6)	0.11	0.017–0.45	2.58 ± 2.03	2.10 (1.8)	1.8	0.93–9.5
1938–1963, no access	26	0.35 ± 0.40	0.21 (3.0)	0.20	0.019–1.9	2.51 ± 1.70	2.04 (1.9)	1.8	0.73–6.4
Male	7	0.65 ± 0.61	0.41 (3.0)	0.48	0.046–1.9	2.92 ± 2.04	2.26 (2.1)	2.3	0.78–5.9
Female	19	0.24 ± 0.18	0.16 (2.6)	0.13	0.019–0.62	2.39 ± 1.50	1.95 (1.8)	1.8	0.73–6.4
1982–1994, no access	20	0.10 ± 0.10	0.06 (2.6)	0.07	0.009–0.41	2.43 ± 1.34	2.06 (1.9)	2.2	0.34–5.0
Male	6	0.12 ± 0.07	0.10 (1.7)	0.12	0.009–0.22	2.27 ± 1.57	1.74 (2.3)	2.2	0.34–5.0
Female	14	0.09 ± 0.11	0.05 (2.8)	0.05	0.018–0.18	2.49 ± 1.30	2.19 (1.7)	2.2	0.95–4.8
1938–1994, all samples	82	0.22 ± 0.26	0.13 (2.9)	0.14	0.009–1.9	2.81 ± 2.42	2.22 (1.9)	2.0	0.38–15
Male	26	0.32 ± 0.38	0.23 (2.3)	0.22	0.009–1.9	3.59 ± 3.41	2.57 (2.3)	2.3	0.38–15
Female	56	0.17 ± 0.16	0.10 (2.9)	0.11	0.017–0.62	2.48 ± 1.69	2.08 (1.8)	1.9	0.73–9.5

^aArithmetic mean \pm standard deviation.

^bGeometric mean (geometric standard deviation).

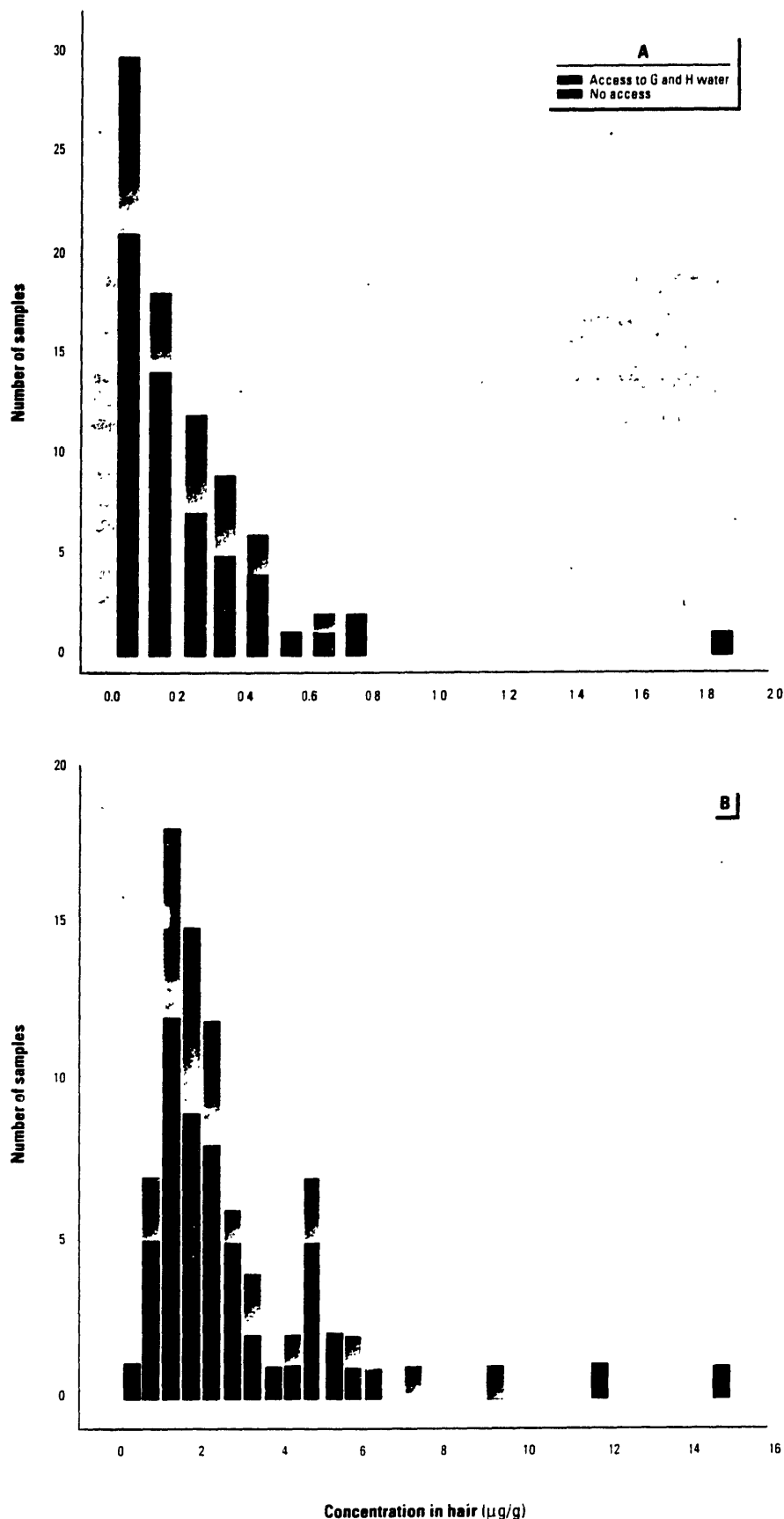


Figure 4. Histograms of (A) As and (B) Cr concentrations in hair samples from Woburn residents.

have access to water from wells G and H were 0.22 and 2.82 µg/g, respectively; the mean concentrations in residents who did have access was 0.21 µg/g for As and 2.80 µg/g for Cr. While there are differences in the populations reported on in the literature (summarized in Table 3), as well as analytical techniques used, it is generally reported that the background, or normal, level in human hair (i.e., the level in healthy individuals who have no known exposures) for As is <1.5 µg/g [the threshold for abnormal ingestion is thought to be 3 µg/g (19)]. This suggests that the levels of As measured in the hair of Woburn residents were within the normal background range, except for one sample which measured 1.9 µg/g. Background, or normal, levels for Cr in human hair have not been reported.

Previous studies have investigated the relationship between access to water from wells G and H and effects on human health. In response to indications that cases of childhood leukemia in Woburn were significantly elevated from 1964 to 1983, Lagakos et al. (35) carried out an epidemiological study and found that about half of the excess leukemia cases were positively associated with access to water from wells G and H. However, access to water from wells G and H could not explain all of the excess leukemia cases because several cases were in the western part of Woburn, which received its drinking water from a different source. In a second epidemiological study, the Massachusetts Department of Public Health (28) reported positive statistical associations between childhood leukemia and access to water from wells G and H if either the developing fetus had access to the well water or the mother had access for 2 years prior to conception. No association was found between childhood leukemia and water from wells G and H for access that occurred between birth and the diagnosis of the disease.

In both of these studies, access to water from wells G and H was estimated by using records of well pumping rates and models of the Woburn water distribution system. Lagakos et al. (35) used a model developed by Waldorf and Cleary (36); the Massachusetts Department of Public Health (28) used a later model developed by Murphy (14). Although the two models indicate that roughly the same area of Woburn received water from wells G and H, the Murphy model (14) indicates a greater temporal variation in the amounts of water received within individual subunits of this area. The Murphy model was developed using updated information on the city's water distribution system, and it was calibrated with pressure and chemical tracer measurements; therefore, it is likely

to be the more accurate of the two models. In our study, we also used the Murphy model (14) to estimate access to water from wells G and H. While we did not find an association between access to water from wells G and H and the concentrations of As and Cr in hair, our results do not contradict or otherwise bear upon the findings of Lagakos et al. (35) and the Massachusetts Department of Public Health (28). Our results merely indicate that Woburn residents who had access to water from wells G and H were no more likely to accumulate As and Cr in their hair than those who did not.

There is clear evidence that wells G and H were inducing recharge from the Aberjona river and that the river water contained elevated concentrations of As and Cr [as much as 70 and 240 µg/l, respectively (13)]; therefore, we were surprised not to have found evidence of exposure to As and Cr in the hair of Woburn residents. One possible explanation for our findings is that the concentrations of As and Cr estimated to have been present in the well water were in fact lower. We have recently found that the peatland (which separates the Aberjona river from the aquifer that supplied water to wells G and H) contains significantly elevated concentrations of As and Cr (37), indicating that these peat deposits trapped As and Cr in the infiltrating river water. If sorption to the peat were a major sink for As and Cr in the infiltrating river water, then our estimates of As and Cr levels in wells G and H water would have been too high. In the case of well H, it was also shown that sand lenses that permeate the peat act as preferential flow paths and probably would not have appreciably removed As and Cr from the infiltrating river water. Nonetheless, these sand lenses are insufficiently conductive to explain the large influx of river water into the aquifer during pumping; therefore, other flow paths, which may or may not be sorptive for As and Cr, must exist.

The possibility that the concentrations of As in the well water were lower than our hypothesized values also seems to be supported by the literature [Table 4 (21–24)]. Valentine et al. (21), Zhang et al. (22), and Khorvar (23) report that significantly elevated concentrations (5- to 10-fold higher than the controls) of As were observed in the hair of people consuming drinking water containing in excess of 100–200 µg/l As. In light of these studies, the maximum amount of As (70 µg/l) hypothesized to have been in water from wells G and H should have been high enough to cause discernible increases in the amounts of As incorporated in the hair of Woburn residents who drank this water.

Only one study relating levels of Cr in hair to concentrations in drinking water was

found. Rosas et al. (24) found that residents of Lecheria (central Mexico) exposed to 900 µg/l of Cr in their drinking water had Cr levels in their hair of 5.1 ± 4.3 µg/g (arith-

metic mean \pm SD), while the control population in Mexico City, which drank water with an average of 20 µg/l Cr, had Cr levels in their hair of 0.68 ± 0.50 µg/g (arithmetic

Table 3. As and Cr concentrations reported in human hair

Samples	Arsenic			Chromium			Reference
	Number	Concentration (µg/g)	Range	Number	Concentration (µg/g)	Range	
Review of a variety of studies	—	—	0.083–96 ^a	—	—	0.09–33 ^b	(19)
Review of selected studies	—	—	0.13–3.71 ^c	—	—	0.13–3.65 ^d	(29)
Diverse population	—	—	0.016–0.61 ^e	—	—	0.23–1.02 ^e	(30)
Diverse population	228	0.10 ± 0.08^f 0.084 (1.9) ^g	0.008–0.67	121	0.82 ± 1.66^f 0.56 (2.0) ^g	0.15–16	(31)
Rural and nonindustrial	80	0.01 (1.32) ^g	—	90	0.21 (1.13) ^g	—	(32)
Diverse population ^h	234	0.08 (1.9) ^g	—	34	0.58 (2.0) ^g	—	(33)
Urban/suburban	37	0.11 ± 0.10^f 0.08 (2.2) ^g	0.02–0.43	—	—	—	(34)
Urban, no known industrial exposure	1,250 ⁱ	0.65 ± 0.70^f 0.46 (2.28) ^g	0.02–8.17	—	—	—	(17)
Tannery workers	—	—	—	71	0.59 ^j	0.31–1.08	(18)
Control subjects	—	—	—	53	0.12 ^j	0.079–0.199	(18)
No access to well water	55	0.22 ± 0.30^f 0.13 (3.0) ^g	0.009–1.9	55	2.82 ± 2.59^f 2.19 (2.0) ^g	0.34–15	This study
Access to well water	27	0.21 ± 0.16^f 0.14 (2.6) ^g	0.017–0.63	27	2.80 ± 2.08^f 2.29 (1.8) ^g	0.93–9.5	

^aRange of mean values reported in 29 different studies; studies include acutely exposed populations.

^bRange of mean values reported in 22 different studies; studies include acutely exposed populations.

^cRange of mean values reported in 15 different studies involving normal (healthy) sample donors.

^dRange of mean values reported in 11 different studies involving normal (healthy) sample donors.

^eRange of mean values for samples from five countries: Japan, India, Canada, United States, and Poland.

^fArithmetic mean \pm standard deviation.

^gGeometric mean (geometric standard deviation).

^hHair donors were selected for lack of occupational exposure.

ⁱSamples were not washed prior to analysis.

^jMedian concentration.

Table 4. Arithmetic mean concentrations of As and Cr reported in human hair in relation to levels in drinking water

Location	Concentration in hair (µg/g)			Concentration in drinking water (µg/l)			Reference
	Number	Mean \pm SD	Range	Number	Mean \pm SD	Range	
Arsenic							
Edison ^a	35	1.16 ± 0.80	0.36–4.31	16	393 ± 31	328–462	(21)
Fallon ^a	45	0.57 ± 0.45	0.08–2.42	23	98 ± 2.2	95–105	
Hidden Valley, NV	21	0.50 ± 0.37	0.01–1.39	10	123 ± 16	107–163	
Virginia Foothills, NV	25	0.48 ± 0.44	0.07–0.44	17	51 ± 13	33–76	
Fairfax, NV	10	0.15 ± 0.11	0.04–0.39	17	<6.0	—	
Xinjiang, China	90 ^b	5.17	—	—	580	—	(22)
	90 ^c	3.25	—	—	580	—	
	83 ^d	0.71	—	—	3	—	
Hungary ^e	22	3.81	—	—	—	>250	(23)
	47	1.78	—	—	—	50–250	
	110	1.63	—	—	—	10–50	
	80	0.59	—	—	—	<10	
Chromium							
Lecheria ^f	93	5.1 ± 4.3	1.10–21	—	900	21–1100	(24)
Mexico City	89	0.68 ± 0.50	0.15–2.76	—	20	1–30	

SD, standard deviation.

^aA community in Bakersfield, CA.

^bSamples were taken from people who had endemic arsenism.

^cSamples were taken from people who did not have endemic arsenism.

^dSamples from control population.

^eSamples were collected in a region of central Hungary between the Danube and Tisa rivers.

^fCity in central Mexico.

mean \pm SD) (Table 4). The amount of Cr hypothesized to have been in water from wells G and H, 240 $\mu\text{g/l}$, and the mean concentration of Cr in the hair of Woburn residents who had access to this water, ~ 2.8 $\mu\text{g/g}$, are consistent with the relationship between Cr in drinking water and Cr in hair reported by Rosas et al. (24). However, as we have already noted, the concentration of Cr in the hair of Woburn residents who did not have access to the well water (and thus, presumably, were exposed to substantially less Cr via drinking water) was also on average ~ 2.8 $\mu\text{g/g}$. Furthermore, the concentrations of Cr in hair did not change as a function of access. Therefore, it appears that access to water from wells G and H cannot explain Cr levels measured in the hair of Woburn residents.

Another possible explanation for our negative findings is that our analysis lacked adequate temporal resolution. If the As and Cr concentrations to which a hair sample donor were exposed were temporally variable, a more powerful analysis could be achieved by measuring the axial distribution of As and Cr along individual hair strands. This approach has in fact been used by forensic scientists to distinguish chronic and acute As and Cr exposures (38–40). By contrast, our analysis of whole, unsegmented hair strands does not detect concentration spikes along the strand.

There are several other sources of uncertainty associated with our methods that could also bear upon our results. The period of hair growth was overestimated for 14 of the samples grown between 1964 and 1979 because the month that the sample was cut was not known. The water distribution model used to estimate access is not entirely free of error. In addition, As and Cr concentrations in the hair samples could have changed during storage. While it is important to acknowledge that these (and possibly other) sources of uncertainty were inherent in our methods, we do not expect that greater knowledge of any of these uncertainties would significantly alter our findings.

Although we did not find evidence of increased As and Cr accumulation in the hair of Woburn residents who had access to water from wells G and H, we did observe that As concentrations in the hair samples have changed over time. The plot of As concentrations in hair samples versus the year that the samples were cut (Fig. 3A) shows that concentrations of As in hair have decreased over the last half century. The concentrations of As in hair averaged over the periods 1938–1963, 1964–1979, and 1982–1994 [expressed as geometric means (GSD)] were 0.21 (3.0) $\mu\text{g/g}$ ($n = 26$), 0.15 (2.4) $\mu\text{g/g}$ ($n = 36$), and 0.06 (2.6) $\mu\text{g/g}$ (n

$= 20$), respectively (Table 2). The reasons for this trend are not known; however, replacement of As-based pesticides by synthetic organic chemicals has undoubtedly reduced the amount of As to which people are exposed through diet and use of pharmaceutical products, and improvements in air pollution control technology have reduced the amount of As emitted into the atmosphere. Also, once the major producers of As-laden wastes ceased operating in Woburn (around 1930), natural attenuation processes, such as stabilization of As-containing particles by vegetation and deposition and burial in pond and lake sediments, may have acted to substantially reduce the amounts of available As to which residents of Woburn are exposed.

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